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For quality of life

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Breeding crops to reduce micronutrient malnutrition

The quality of diets has received considerable attention in global development policies, complementing the traditional focus on protein and energy consumption. Deficiencies, particularly of iron, zinc, iodine and vitamin A, limit the well-being and productivity of over 2 billion people worldwide. Micronutrient malnutrition is primarily a symptom of poverty and thus tends to decline with improvements in livelihoods. Targeted strategies available to alleviate micronutrient deficiencies include dietary diversification, food fortification, supplementation, and more recently breeding of agricultural crops for higher nutrient levels (or 'bio-fortification').

Agricultural diversification combined with nutrition-awareness can lead to *food diversification*, especially in rural areas although the success of such programmes in improving nutritional status has been mixed. *Fortification* is possible with a limited number of micronutrients and in foodstuffs that target groups purchase. This strategy, common in many industrialised countries is better suited to reaching the urban poor and is more difficult in subsistence situations. In such circumstances, salt and sugar may be the only purchased products, offering important but limited opportunities for fortification: salt with iodine, sugar with vitamin A. *Supplementation* can be targeted very well, but requires a logistical infrastructure for distributing tablets/capsules and ensuring their proper use. Supplementation is thus easier for compounds that the body can store, such as Vitamin A, than with micronutrients that need to be distributed at a much higher frequency. Supplementation is a necessary strategy for targeting particular groups (e.g. pregnant women), being most effective when using existing health care facilities, such as prenatal services in primary health care clinics. Biofortification, a recent addition to this basket of strategies, consists of increasing the availability of micronutrients in staple foods, through breeding strategies.

Each of the available strategies for combating micronutrient malnutrition has its advantages and limitations. Nutrition experts generally agree that combinations of different approaches need to be formulated according to specific circumstances. A scarcity of public funds for this important area means that the relative roles of the various strategies need to be weighed up carefully. This brief concentrates on the potential of biofortification to play a role in addressing micronutrient deficiencies and summarizes the key technical and policy issues.



Box 1. Cost comparisons: the case for biofortification

Financing of nutrition interventions is divided among supplementation, fortification and food-based approaches. Debates continue among nutrition specialists about the mix of strategies to be prioritized under different circumstances (Allen and Gillespie 2001). The proponents of biofortification claim that the economic arguments for investing in this area are clear. Cost-benefit ratios of 21 or higher have been proposed (Bouis 1999) which, even though conservative, are two to three times that of other nutritional interventions. Biofortification should however be seen as a long-term strategy, only yielding results at least 10 years from now. How should policy makers in developing countries and donor organisations view such impressive numbers? Biofortification has to reach additional target groups and/or offer cost advantages with respect to similar strategies for it to be a worthwhile investment. Estimates of unit costs of fortification and supplementation of vitamin A and iron are provided in the table together with initial estimates for iron biofortification. The numbers illustrate the case to be made for investing in biofortification in terms of cost effectiveness.

Table of estimated unit costs of micronutrient interventions

Micronutrient	Unit cost (\$)		
	Supplementation	Fortification	Biofortification
Iron	1.70 / pregnancy	0.09 / person / year	0.015 / case reached 0.008 person / year
Vitamin A	0.20 / child under 5 / year 0.50 / person / year	0.05 – 0.15 / person / year	?

Sources: supplementation and fortification: Allen and Gillespie (2001), Horton (1999) based largely on actual interventions; biofortification: estimates by CIAT and IFPRI (2002)

The cost argument is also bolstered by the potential to improve the micronutrient status of malnourished groups largely not reached by national-level fortification programs. The low unit cost estimates for biofortification reflect the scale benefits possible with breeding strategies. Once a micronutrient-enriched variety has been bred, the marginal costs of delivering it are relatively minimal in comparison to fortification or supplementation alternatives. This is the essence of the “sustainability” advantage of this approach, although it is recognised that continued adaptive breeding will remain necessary.

Supporting biofortification at this point still amounts to investing in research, both in breeding and bioavailability studies. If this investment takes place, then more reliable information will become available over time concerning availability to specific target groups and nutritional impact. Eventually this should help countries to design even more effective combinations of nutrition intervention programmes, and ultimately to improve the lives of billions. A fiscal difficulty is, of course, that funds currently made available for nutrition interventions are far from sufficient in comparison to the problem at hand, despite the solid economic arguments for these social investments. This further exaggerates the problems faced in allocating scarce public resources when technological developments open up new possibilities.

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1. Economic and sustainability issues

The various micronutrient strategies differ in their costs and timeframes. Food-based approaches have a medium to long term perspective. Supplementation is relatively expensive, mainly due to the logistical costs of reaching the rural poor. Fortification works best at improving micronutrient status of the overall population, as opposed to acute cases. Both strategies can be effective at short notice, but require a continuous financial commitment. Not only are there sustainability and targeting concerns with these approaches, but most governments and international donors have yet to commit sufficient capacity or cash.

For this and other reasons, long-term food-based approaches, have also received attention. There is some experience with crop and dietary diversification programmes, particularly where these are linked with extension programmes for improved nutrition. But mainstreaming of such approaches beyond the community level has not been successful as yet and requires further work.

Preliminary analysis indicates that relative costs of biofortification offer some interesting opportunities (see Box 1). Although this requires a long-term investment over 10-15 years in pre-breeding, once high-quality breeding materials are available, the additional cost to increase the nutrient content in normal breeding programmes will be minimal. After this initial investment, the strategy may thus be considered to be more sustainable, but it cannot be expected to contribute to reducing micronutrient deficiencies for 10 years to come.

CGIAR scientists point out though that these initial investments should be put in perspective. They estimate the costs of performing the required pre-breeding operation for increased zinc, iron and vitamin A availability in six main staple crops at less than US\$100 million. This corresponds to approximately the costs of vitamin A supplementation of 100 million women and children in South Asia for just two years, which is arguably one of the most cost-effective supplementation approaches.

2. Breeding for nutrition

Plant breeding has proven to be a very powerful tool to increase yields, especially in environmentally favourable conditions. Increasing total production in South and Southeast Asia in the 1970s and 1980s, however, went hand in hand with increased numbers of urban and rural poor, reduced agro-biodiversity and dietary diversity, and reduced micronutrient contents in the food crops. More recently, strategies have been adapted to address these limitations. Specific breeding programmes for micronutrient-content have, however, been limited. This was partly due to a lack of contact between breeders and nutritionists, and also because of methodological limitations.

Breeding for nutrient content has to deal with the following issues:

- Is sufficient variation available within the crop species for cross breeding, or can the right variation be induced through mutations
- Is the heritability of the valuable traits high enough to warrant effective selection
- Are effective and efficient tools available for screening plant populations for **desired traits**
- Can higher nutrient content be combined with increased yield and yield stability?
- Can higher nutrient content be combined with acceptable consumption qualities (taste, colour, cooking quality, etc.)
- Can breeding for nutrient quality be combined with breeding for diversity.

When opportunities are limited in conventional breeding, transformation (genetic modification) may offer additional opportunities. In that case some additional issues have to be taken into account:

- Food safety of the additional constructs used in transformation
- Environmental safety of the transgenic crops
- Ethical acceptance of the technology and product in the communities concerned
- Effects of intellectual property rights on ownership over the research agenda and its output
- Effects of transgenic products on export opportunities.

Box 2. The potential to improve the nutrition value of cereals by breeding

For many people living in developing countries, rice is their major energy and nutrient supply. Unfortunately the rice they consumed currently, i.e. the milled and well-polished white rice, is a product with satisfactory appearance, but most of the nutrients are lost during milling. The major nutritional deficiencies of typical rice-eaters are in vitamins and minerals. Sudden death both in adults and infants caused by cardiac failure (beri-beri disease) is the best-known deficiency caused by a shortage of vitamin B1. This disease became widely spread through rice-eating regions of Asia after the truncated-cone type rice mill was introduced in early last century.

Transgenic approaches have been taken to improve the contents of certain micronutrients such as vitamins and iron in seeds. For example, the "Golden Rice" generated in Prof. Ingo Potrykus' lab is to express 3 genes in vitamin A biosynthetic pathway in rice endosperm, which leads to the production of 1.6ug/g pro-vitamin A, β -carotene, in the rice grain (Ye et al, 2000). Traditional, i.e. non-GM, breeding can potentially improve the nutrition value of cereal crops by 1) improving the content of nutrients in the grain and 2) by increasing the bio-availability, particularly by decreasing certain anti-nutrition factors such as phytate. The following aspects need to be considered when such approaches are considered:

- 1) The impact on yield (to plant growth and seed viability)
- 2) The genetic possibility (genes and traits available in the species)
- 3) The public acceptance (the taste and appearance)
- 4) The relative long-term investment

Technically there are many possibilities to improve the nutrient quality of cereals. For example, in rice the natural variation in Fe contents varies from 6.3ug/g to 24.4ug/g, and in Zn from 13.5ug/g to 58.4ug/g. This give a 4-fold difference between different varieties (Gregorio 2001). Screening for low phytate mutations has been carried out in several crop species (Raboy, 2001, 2002). Most seed phytate is deposited as mixed phytin salts of mineral cations such as K, Mg, Fe, and Zn in the form of microvacuoles or protein bodies (globoids). Loss-of-function mutations of two loci in corn, *low phytic acid 1-1 (ipa1-1)* and *low phytic acid 2-1* were found to confer seed phenotype. The former one gives 75% reduction of phytate and the latter one gives 50% reduction. More than 20 alleles have been found in the *ipa1* locus, conferring 50% to 95% reduction of seed phytate (Raboy et al., 2001). Similar mutations have been found in barley, rice and soybean (Raboy 2001, 2002). Studies have showed that 75% reduction of phytate gives no phenotype to plant growth and yield, however, 90% or more reduction has more severe impact on seed and plant growth (Raboy 2001). Small clinical trials on human have showed that the *ipa1-1* corn could lead to 50% more iron absorption and 76% more Zn absorption (Mendoza et al., 1998).

A different approach (currently being investigated by one of the authors together with colleagues at Plant Research International) entails modifying the rice seed structure and storage product content to prevent the nutrition loss during milling, aiming at improving the nutrition value of rice in general. This may provide a 'short-cut' route to improve a range of naturally occurring micronutrients in rice.

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Furthermore, the products of breeding have to:

- Reach the target groups and be acceptable for them
- The bioefficacy of the additionally ingested nutrients has to be high, i.e. the chemical composition and the matrix have to allow for effective absorption and conversion in health-promoting forms.
- The increased consumption should not lead to toxicity.

3. Opportunities and limitations

Micronutrients

Iron and *zinc* in the diet has been extensively researched. Approximately half of the iron and zinc intake in Asia is accounted for by the staple crops, rice and wheat. Doubling the content (and bioavailability) in these staples can potentially solve the majority of deficiencies without changing the food habits. Uptake of iron and zinc by plant roots do not change the colour or taste of the product and may even contribute to increased yield. There is a significant genetic variation in genebank collections, and heritability is sufficiently high to expect considerable success in conventional breeding. While critics of this strategy raise concerns about the number of obstacles for breeders to overcome, the use of molecular markers is likely to speed up the selection process considerably.

Iodine is in short supply in almost all soils. Opportunities to increase uptake through roots into edible parts of plants is therefore not a serious option. Fortunately, iodine fortification of salt is a relatively cost-effective, and tested, strategy. However, greater investments in capacity and monitoring are still required.

Apart from increasing the content of the micronutrients, additional opportunities include other strategies to increase the bioavailability of the existing nutrient content. Iron and zinc availability can be increased, for example, by reducing phytate content (see Box 2).

Vitamins

Vitamin A is considered to be the major vitamin deficiency in many parts of Asia, Africa and Latin America. Pro-vitamin A (beta/alfa carotene) is present in most yellow and red fruits and vegetables.

Bioefficacy is however low, which is mainly due to the low conversion rate to the biologically active compound retinol. Genetic diversity in germplasm for carotene in staple crops is very low, and success through conventional breeding is not expected. A positive exception is orange fleshed sweet potato. Transgenic approaches may contribute to increase the supply of vitamin A through staple crops.

Breeding strategies

Where particular genes and promoters that code for the production of certain compounds cannot be found in the crop species, transformation may create new opportunities. This is for example the case with genes from the Daffodil that code for the production of carotene in rice ('Golden Rice'). Basically the whole plant and animal kingdoms are available for supplying new traits to crops. This potentially opens up a wide array of new opportunities.

Recently a potato with high methionine content has been produced in India using *Amaranthus* genes. Similar results have been obtained using the vast genetic knowledge developed with the model plant *Arabidopsis*. Even though the use of transformation is technically feasible in many crop species, its use is widely debated (see Box 3)

4. Targeting breeding

Major concerns have been aired about the feasibility of breeding for nutrition, especially with regard to combining high-nutrition varieties with resource-poor farming (see Box 3).

Breeding for benign agro-ecological conditions is relatively easy since many environmental and biotic stress factors are less important than in less favoured areas, partly due to the use of inputs such as fertilisers and irrigation. Therefore, breeding for reducing nutrient deficiencies for the urban poor may be relatively straightforward. In general, the farmers who supply Asian cities cultivate in such relatively benign conditions, are using so-called improved varieties for their market crops. They can be approached relatively easily to replace their varieties with high nutrition-seeds. However, they will do this only if the market is ready to take up the product and if the added characteristic is built in a variety that responds well to their farming conditions.

Box 3. Golden Rice

Golden Rice is the result of publicly-funded research to induce rice to produce beta-carotene in the endosperm of its seeds. Its announcement caused a lot of turmoil in 2000, the first public debate on biofortification. The main arguments are listed here.

Promise: An estimated 124 million children get insufficient vitamin A, causing irreversible blindness in 500,000 cases each year. Lack of a balanced diet, especially during dry seasons (when vegetables and root crops are expensive) due to poverty or ignorance is the main reason. Biofortified rice, the staple of most of these children, could prevent many cases of blindness and even death. Golden Rice produces retinol, a precursor of Vitamin A; new strains that also contain bioavailable iron and zinc are being worked on.

Technical comments: Golden Rice is a genetically modified rice with genes from daffodil and a bacterium inserted. Opponents consider transgenics by definition unstable, a problem that is significant in Golden Rice because of the complexity of the insertions and the use a CaMV-promoter. Furthermore, GMOs are suspected of carrying risks of environmental safety (e.g. horizontal gene transfer) and food safety. Apart from beta-carotene, also other compounds may be produced in the endosperm with unknown nutritional effects, and the use of antibiotic markers has been criticised (Institute of Science in Society: www.i-sis.org.uk/rice.php).

Nutritional value: Opponents claim that too little beta-carotene is produced and there is insufficient knowledge about the bioconversion factor of this into Vitamin A. Greenpeace claims that adults need to eat an impossible amount of 3.7 kg of dry-weight rice daily to obtain the needed amounts (<http://archive.greenpeace.org/geneng/highlights/food/goldenrice.htm>). In case of severe malnutrition (lack of fats and iron in the diet) or diarrhoea, bioconversion levels will drop even further. The inventors, however, claim that amounts of retinol will significantly increase in the next generation of Golden Rice, and that it is intended to supplement, rather than be the sole supplier of pro-vitamin A.

Targeting and acceptance: Will it be possible to convince farmers to grow the rice e.g. remote small-scale farmers whose families are at constant risk? Will consumers abolish the general preference for pure-white rice? And if they do, would it not be better to stimulate the use of brown (unpolished) rice? Furthermore, Golden Rice will allegedly further reduce agro-biodiversity when a small number of modern 'golden' varieties will leave the research stations, targeted at remote areas where genetic diversity in the rice crop is still significant.

Expensive 'Golden Bullet': Other opponents say that the amount of money involved in the research could have better been used in other Vit. A programmes such as supplementation or food diversification. This raises the difficult issue of prioritising such funds between approaches that target current or future problems. Golden Rice is also considered a typical example of a reductionist approach to addressing nutritional deficiencies, that does not tackle the underlying causes: poverty and monoculture.

PR-campaign: Golden Rice is seen cynically by opponents of all genetic modification as "a Trojan horse", part of a deliberate exercise to make the (GM-) technology acceptable (www.genepeace.ch/new/fields_of_dreams.htm). The licensing of the rights to Syngenta under the promise to provide royalty-free access to small scale farmers is viewed suspiciously as simply a PR-campaign by the life science multinationals who or not to be trusted. In this view, Golden Rice will also serve to introduce the concept of intellectual property, which is alien to rural communities. The NGO 'GRAIN' furthermore claims in addition that Syngenta has been given control over the results of publicly funded research almost for free. (www.grain.org/publications/delusion-en.cfm).

The inventors have to deal with this broad array of opposition. From a comment by Dr. Potrykus' : "there is a need for distribution, fortification, dietary diversification and education. All of these are important. These interventions have used an impressive amount of funds that have been spent over the last twenty years and have been very helpful. But we still have 500,000 blind children and millions of Vitamin A deficiency deaths every year." (www.fumento.com/goldenrice.html).

Our comment: Most of the opposition to Golden Rice is based on a blanket rejection of genetically modified crops. However even a willingness to accept GMOs still leaves a number of issues, such as targeting in relation to cost-effectiveness, which are also relevant for other (i.e. non-GMO) biofortification initiatives. It may be golden, but it is no silver bullet.

Breeding for resource-poor farmers is more difficult. Their farming conditions vary in time and place, requiring either various different varieties, or varieties with high plasticity, adapting to various stresses. Furthermore, delivery systems of seed for these resource-poor farmers are less developed. The result is that the use of genetically diverse landraces, without scientific breeding improvements, is common among these farmers.

This creates a major challenge for breeders who want to target malnutrition among these remote and resource-poor farmers.

The technical question is how to build the new characteristics in such a wide genetic base that varieties or mixtures can be selected for every niche.

The socio-economic question is how to make sure that such farmers see the benefit of growing and consuming it.

The environmental question is how to avoid the introduction of high-nutrition crops causing a major reduction of the agro-biodiversity that resource-poor farmers are currently preserving.

The nutrition question is how to ensure that the further adoption of such modern varieties does not come at the expense of secondary, potentially more nutrient-rich crops.

5. Conventional or transformation?

The choice of the breeding method has an influence on the opportunities to tackle these questions. Conventional breeding will require a very long-term investment, especially when the characteristic can only be found in genetically distant plant materials. Crossing and subsequent generations of backcrossing under continuous selection is required to bring a new characteristic into a genetic background that is suitable for farming. It is time-consuming to do that for one variety (or set of related varieties); it is extremely laborious to perform the same for a wide variety of populations that may be needed to select materials for the diverse needs of resource-poor farmers. The use of marker-assisted selection can partly solve this concern.

The opportunities for mutation breeding have not yet been fully mapped. However once a useful trait has been identified using mutation breeding, it could likely be repeated with widely differing populations, potentially creating diversity in the high-nutrition crops relatively quickly.

The use of transgenics may be the quickest way to introduce a new characteristic in the preferred genetic background. However, when multiple genes, promoters etc. have to be introduced, the cost may become quite high. Furthermore, insufficient experience has yet to be obtained with the stability of introduced genes with a high expression: gene silencing commonly reduces the effectiveness of the introduced genes.

Whatever the breeding technology, a significant role has to be given to the farmers themselves in developing materials that are optimally suited to their specific conditions. This required a new impetus to the development of new methodologies for participatory research and seed system development.

6. Conclusion

Breeding to improve the low nutrition status of the poor faces all the challenges that other breeding initiatives have. These are particularly complex when remote rural poor have to be targeted. Biofortification offers more immediate potential for reducing micronutrient deficiencies of those who were also the principal caloric beneficiaries of the Green Revolution. Considerable gains may be possible at a cost-effective manner when compared to other strategies to combat micronutrient deficiencies. Despite the fact that these are long-term strategies, they arguably deserve research investments.

However, the remarkable lack of impact of the diffusion of modern varieties in "less favourable conditions", such as much of Sub-Saharan Africa, may well be repeated here. These ongoing legacies and realities should not be forgotten in the rush to embrace new technological opportunities.

Box 4 Example of a research programme: “Food-based interventions to alleviate micronutrient deficiencies: A food chain approach.”

Breeding for micronutrients does not just mean to “construct” a cultivar with all desired traits. Thorough knowledge about genotype-environment-management (G x E x M) interactions is needed to make sure that genetic make-up leads to the desired outcomes e.g. higher bioavailable zinc and iron in cereal grains. Insight in post-harvest storage and food preparation is also needed to avoid that all that is gained through breeding is lost during the transformation from grain to food. Furthermore scarcity of monetary and manpower resources urges decisions as to the best level of interference in terms of absolute gain and in terms of relative output/input. Choices need to be made between breeding for higher micronutrients, breeding against anti-nutritional factors (ANF), post-harvest treatment to deactivate anti-nutritional factors, and nutritional gains against yield losses, etc.

A food chain approach is used in an ongoing research programme at Wageningen University as a means to investigate the possibilities and relative impact of interventions at different levels on micronutrient supply. This approach can at the same time serve to position breeding for micronutrients in relation to other interventions.

In partnership with universities and research institutes in Benin, Burkina Faso and China the food chain approach is researched on two staple foods: sorghum and aerobic rice. The partners were very clear that improvements leading to even minor yield losses would not be acceptable. Ten staff members of the partner institutions are involved as PhD students to investigate part of the chains.

In West Africa, soil and water conservation measures and phosphate fertilisation are implemented to boost yields. The impact of these measures on zinc and iron uptake and phytate (ANF) formation is investigated. A breeding strategy for sorghum is elaborated based on chemical and DNA analysis of the core collection of 200 accessions from ICRISAT/CIRAD and field experiments with selected varieties. These two studies take place in Burkina Faso and account for possible G X E X M interaction in an early stage. Food processing strategies related to different sorghum cultivars and desired products, and their impact on zinc and iron content and on phytic acid and tannin content (ANFs) are investigated in Benin. Dietary practices and choices identifying sources of micronutrients and anti-nutritional factors are investigated both in Benin and Burkina Faso. Two intervention studies will be performed: the first comparing two cultivars that differ in micronutrient/ANF ratio, the second comparing products of two food processing methods of the same cultivar. Their impact on the human micronutrient status will be measured. Varieties and food processing methods used are derived from the studies performed in the programme.

Also the programme components in China follow the food chain. The transition from lowland (flooded) rice to upland rice production means a change from anaerobic to aerobic soil conditions. The influence of this change on zinc and iron availability and uptake is investigated. At the same time, aerobic rice varieties are screened on zinc and iron content and on their capability to grow under low and high zinc or iron conditions. The physiological basis behind effective micronutrient scavengers and accumulators etc. is investigated. The place of micronutrients and anti-nutritional factors in the cereal grain is investigated, as this may be the key to successful zinc and iron bioavailability after processing. A dietary investigation is undertaken to identify sources of micronutrients and anti-nutritional factors. In China, zinc-enriched rice achieved through foliar zinc application is available and marketed as “healthy food”. Human nutrition trials are used to investigate whether the claim is justified.

At the end of the program, we expect that possibilities become clearer and informed choices can be made. The inclusion of staff of the partner institutes in the actual research will contribute to capacity building in these institutes on the micronutrient issue. The research results can be used by policy makers to decide on allocation of scarce resources and by research institutes to decide on research priorities. Also the international body of scientific knowledge will have been increased and southern researchers will have gained a position in it.

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