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PLANT BREEDING AS AN EXAMPLE OF 'ENGINEERED' EVOLUTION

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Crop and forage plants which cover most of our cultivated land and provide, directly or indirectly, most of our food, have not in fact, and never would have evolved naturally. Their development required the intervention of man into the natural process of evolution.

Man (plant breeders) have used the principles of evolution – genetic variation, sexual hybridisation combined with subsequent selection of the best adapted offspring to specific ecological niches – in several ways: They have increased the frequency of genetic variation by physical or chemical mutagenic treatment. They have encouraged novel genome combinations and recombinations within and across species barriers by intraspecific hybridisation (within species) and by interspecific hybridisation (between species). In those cases where interspecific hybrids were not viable, they used embryo rescue techniques (in vitro culture of otherwise abortive embryos). They transferred random parts of genomes by fertilization with partial nuclei, which they recovered from cells treated so badly that the genome was totally fractionated into random pieces They grew haploid plants from germ cells to achieve rapid homozygocity and they used the cell poison cholchicin to recover polyploid plants. And they have given evolution specific directions by selecting for traits in their interest, and against undesired traits (see Fig. 1, p. 614).

Taking the example of *Brassica oleracea*, it can be well visualized, how breeders have changed the phenotype (and the underlying genotype) of a plant originally created by natural evolution. *Cabbage* is the result of excessive development of a single *terminal bud* (and suppression the rest of the plant); *Brussels sprouts* were created by selecting for mutations leading to development of miniature 'cabbages' from all *lateral buds*; breeders have created *Kohlrabi* by selecting for mutations leading to *swelling of the basal* *stem*, *Kale* was created by favouring *over-dimensional leaves*, *Broccoli* by collecting mutations leading to *excessive overproduction of flower buds and stems*, and *Cauliflower* by an extreme *overproduction of complex systems of flower buds*. All these cabbage varieties are still the same species (*Brassica oleracea*), but they are totally unsuited for survival in any natural environment, not even for independent propagation, and would, therefore, never have evolved without intervention of man. The difference in their morphology indicates the difference in their genome and there is not the slightest doubt that these genomes have been intensively genetically modified – long before the advent of 'genetic engineering'.

Breeders also combined different desired traits (genes) within the species or across species barriers to develop ever improved varieties to exploit the potential nature has provided. Virtually all plants used to date in agriculture and horticulture are intensely genetically modified in the interest of man. None of these biologically disadvantaged plant varieties would ever have evolved and they would disappear within few generations without the continuous care provided by farmers in the artificial habitats of agriculture (see Figs. 2 and 3, pp. 615-6).

All of our crop plants (including those used by organic farmers) were developed (on the basis of such intense and uncontrolled genetic modification!) and were consumed without any special precaution. And there was no harm to the consumer. Instead mankind was protected from starvation, was enjoying an ever improved supply of foods, and an ever prolonged life.

This adaptation of plants to the needs of mankind is a never ending process, with exponential demands to date, because the exponential population growth requires that agriculture is providing an increasing amount of food. To help save harvests plant breeders are continuously challenged by the natural evolution of pests and diseases, which overcome existing resistance. They have to develop novel varieties which are more resistant to biological stresses exerted by novel pathogens. The same is true for physical stresses decreasing possible harvests. And plant breeders have to work on increasing the potential of crop plants to exploit the natural resources (to increase future harvests). It is mandatory to perform plant breeding with ever increasing efficiency. There is no other option for an ever growing world population of already 7 billion to return to pre-industrial agriculture, where only a privileged minority would have a chance for a decent life.

Thus far, up into the 80s of the 20th century, the approach to man-made evolution was based on *trial and error* and *learning from experience* and knowledge was limited to *phenotypes*. It should be considered very fortunate that, just when demand for more food was becoming an overwhelming problem, progress in science was providing, with the advent of molecular biology and plant cell culture, a refinement and extension of the tools for breeding, enabling to adapt plants to the needs of man with increasing efficiency.

These novel tools are now based on *knowledge and understanding*. Complex phenotypic traits can be analysed on the level of genes, their regulatory signals, and their interactions with other genes in biochemical pathways and cellular networks.

To illustrate the difference between traditional and state-of-the-art breeding let us consider one concrete example: Let us assume we want to develop a vitamin A rice to combat vitamin A-deficiency. The traditional breeding approach would explore whether evolution has created anywhere in the world a wild or cultivated rice variety which has a yellow endosperm (which would indicate the presence of provitamin A). The probability is not very high, because this trait does not offer any selective advantage in any ecosystem. The result was, therefore, not surprising. A careful analysis of the entire worldwide gene pool of rice and its relatives disclosed that nature had not developed a plant which could be used as starting material for a traditional breeding program. Breeders had no possibility to work on the development of vitamin A-rice with traditional methods. And therefore breeders asked for help from the upcoming genetic engineering technology. This state-of-the art approach works as follows: The first step is to find out how pro-vitamin A is synthesized in plants (to understand the biochemistry of the biosynthetic pathway including the metabolic bottlenecks and their regulation). This is followed by the elucidation of the genes involved (including the number of genes, and their tissue-specific expression). Thereafter it is necessary to isolate the genes and regulatory signals. This can be done from rice or from a model plant or a micro-organism if this has such a biochemical pathway. The next step tries to introduce the missing genes together with appropriate regulatory signals (in our case signals which activate the genes in the endosperm cells of the seed storage tissue only) into somatic single cells. As this is a random process which requires millions of cells, it is mandatory to add a gene which enables to built up a tight selective pressure, which allows only those few cells to survive, which have taken up and integrated the genes of interest. From those single cells complete plants are regenerated, which finally produce seeds which transmit the novel genes (and traits) to all offspring (see Figs. 4 and 5, p. 617).

Taking this state-of-the-art approach vitamin A-rice became a reality in 1999. Following the same approach, in principle, any complex phenotypic traits can be analysed at the level of genes, their regulatory signals, and their interactions with other genes in biochemical pathways and cellular networks. Genes for desired traits and appropriate regulatory sequences can be isolated, newly combined, and their function predicted and tested experimentally. They then can be introduced selectively into otherwise unaltered genomes, thus providing *'direction'* for evolution even before selection (see Fig. 6, page 618).

This approach of elucidating the molecular basis for a given trait and of designing the biochemical pathways prior to re-introduction into a target plant, can be applied – in principle – to all those traits of outstanding importance for future food security such as drought tolerance, flooding tolerance, salt resistance, heavy metal resistance, disease resistance, pest resistance, improved nutritional value, reduced toxins, allergens, anti-nutrients, reduced post-harvest loss, improved response to climate stresses, etc. Not only is it possible to introduce desired traits, but it is also to inactivate undesired traits such as anti-nutrients or allergens and traits which are not available in a given species can be introduced from other species. These and other technological possibilities enable breeders to exert 'direction' and to 'predict' novel phenotypes by not only selecting gene combinations from increased variation, but by planning variation and gene combinations 'a priori' and making more efficient use of the potential nature is providing to us.

These improved technological possibilities are urgently needed to secure food for an increasing world population, which will not level off before it reaches 10-12 billion and for which at least twice as much food has to be produced in countries where already now one billion people are starving. And this food has to come from agricultural production systems which are already under tremendous stress because of shortages in land, water, manpower, energy, and capital and which are expected to produce all this additional food with less negative impact onto the environment.

Past progress in agricultural productivity is, of course, not only the success of plant breeding, but of all science and technology invested into agriculture, including mechanisation, synthetic fertilizer, pesticides, insecticides, integrated production, irrigation, expansion of agricultural land, biotechnology, and many more inputs. The challenges ahead will require far more intensive financial investment in further research in all these areas. It would be naive to assume that any one of these contributions alone can solve the burning problems of future food security.

To save the last remaining refuges of natural environments is only possible if we can produce more food on the agricultural land already in use. There is no alternative to intensive and sustainable production systems. And this requires careful exploitation of science and technology and the tools both are developing.

Paradoxically, as long as 'man-made evolution' was based on 'trial and error', without any other knowledge base than 'experience and phenotype' it was considered 'natural' and was accepted by our society. Now where the same is based on 'science, knowledge, predictability, and controlled experimentation', the same process is discredited as being un-natural, highly dangerous, unethical, and unacceptable.

This (typically European) attitude lacks any justification from science, experience, logic, and common sense, but it is a widespread psychological fact, and difficult to change with argumentation based on science and logic. The consequence of this attitude are regulations for the use of the technology, which prevent that the technology is used by public sector institutions, for altruistic solutions in the interest of the poor in developing countries.

Many of the lives lost to starvation and micronutrient deficiency could be saved, if European societies would change their hostile attitude towards this knowledge-based progress in plant breeding technology, which is nothing else but a more sophisticated continuation of the use of genetic modification to the benefit of mankind.

This European attitude is extremely unfortunate for those underprivileged poor in developing countries, for which food insecurity and malnutrition is a question of life and health. As the Nuffield Council on Bioethics phrases it: Europe is ignoring a moral imperative to support use of genetic engineering technology to the benefit of the poor.

Well-fed European societies have forgotten that our agricultural productivity depends upon centuries of exploitation of science and technology and cultivate instead a romantic imagination of farm life which was not even true in 1565, when Peter Breughel painted his popular picture, which represents a dream, not reality. Neither were the farmers well-fed nor the fields so productive and without weeds (see Fig. 7, p. 619).

Millet's picture from 1862 – in contrast to Breughel's – is 'honest' and reminds us that pre-industrial agriculture was, also in Europe, and not so long ago, not at all romantic, but a back-breaking fight for survival – the same way as it is today for hundreds of millions of farmers in developing countries. It is immoral to deny those farmers the help from technology-supported agriculture (see Fig. 8, p. 620).

Use of genetic engineering technology for the adoption of crop plants to the needs of mankind is neither 'unnatural', nor 'unethical', nor 'hazardous', as considered by many European citizens. It is state-of-the-art use of the natural potential. It is at least as safe as previous tools in plant breeding. There is consensus in the scientific community that this technology does not carry any novel inherent risk.

FURTHER READING

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Economic and environmental impacts of agbiotech: a global perspective. By N. Kalaitzandonakes (ed). Published in 2003 by Kluwer-Plenum Academic Publishers.

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Mendel in the kitchen garden: a scientist's view of genetically modified foods. By Nina V. Fedoroff and Nancy M. Brown. Published in 2004 by National Academies Press.

EU Directive 90/219 on the contained use of genetically modified micro-organisms.

EU Directive 90/220 on the deliberate release into the environment of genetically modified organisms.

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Let them eat precaution. By John Entine (ed.), AEI Press, Washington, 2005.

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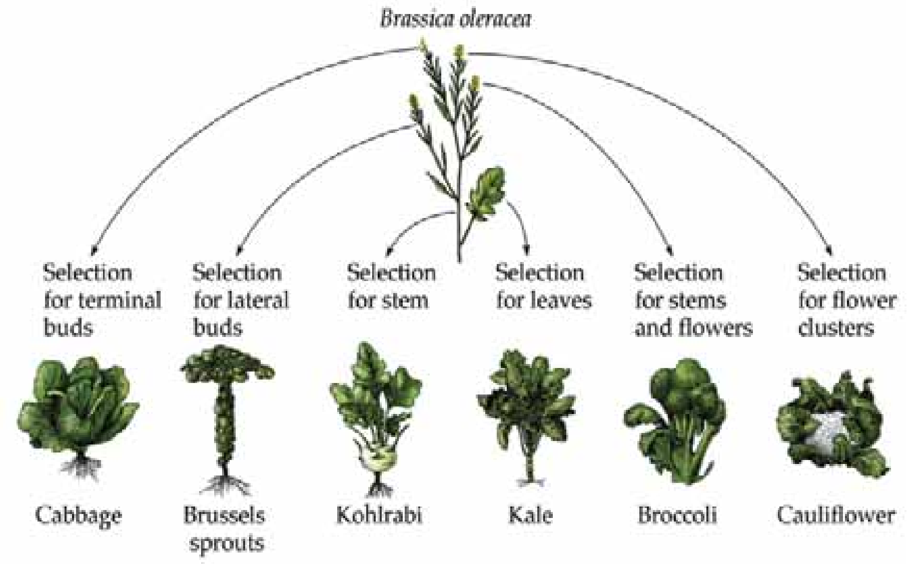


Fig. 1 A variety of different cabbage crops have been bred from wild cabbage, by selecting amongst the genetic variation for different desired traits.

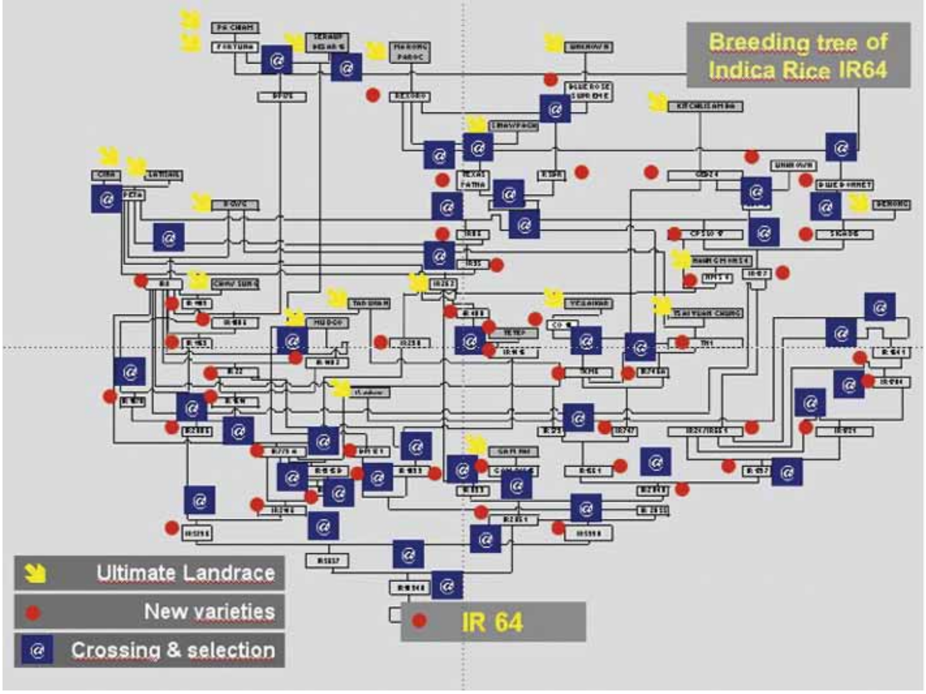
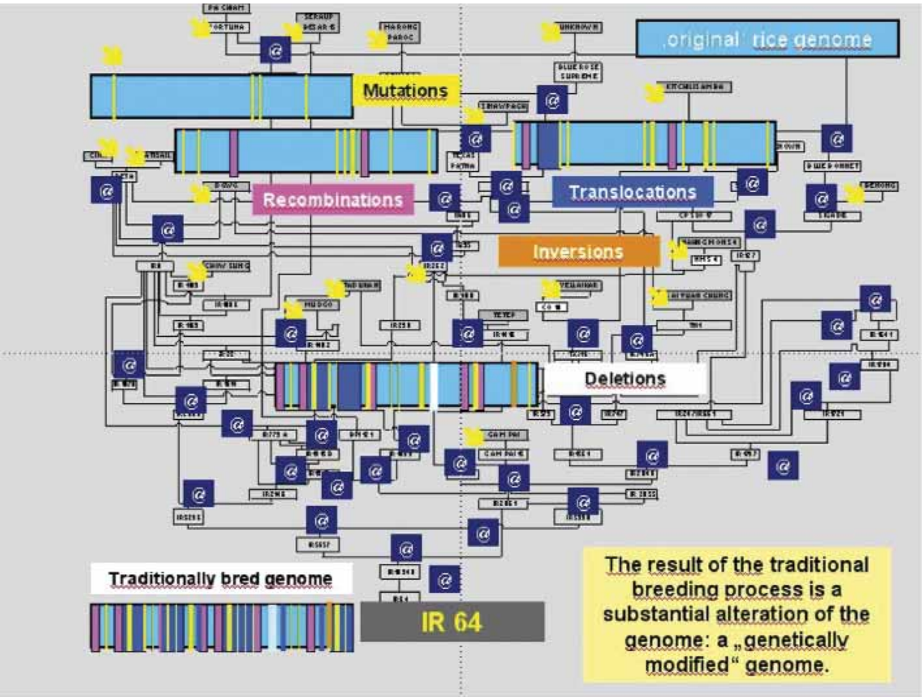


Fig. 2. The 'breeding tree' (the history of the breeding process) of the most popular and widespread rice variety IR64, indicates over how many breeding steps the development of a modern variety evolves. The yellow arrowheads indicate 'landraces' (mutant forms of rice selected by farmers for specific beneficial traits) and the blue boxes indicate the steps of sexual hybridisation and selection. Each of these parameters and steps inadvertently leads to uncontrolled and unpredictable alterations of the genome. And the breeder has no control on these changes but just selects, among hundreds of thousands of offspring, the single one with the desired trait combination.



The result of the traditional breeding process is a substantial alteration of the genome: a „genetically modified“ genome.

Fig. 3. A graphic representation of the types of uncontrolled changes in the genome that accompany the traditional development of a modern crop variety. *Blue* represents the original genome; *yellow* are spontaneous or induced *mutations*; *red* are *recombinations* (rearrangements of large parts of the genome); *blue* are 'translocations' (excision from and re-integration of at novel positions in the chromosome); *orange* are *inversions* (excision and re-integration with opposite polarity of entire chromosme pieces); *white* are *deletions* (loss of entire fractions of chromosomes with hundreds of genes). All these uncontrolled 'genetic modifications' of the genome were the basis for those crop varieties, which we eat daily.

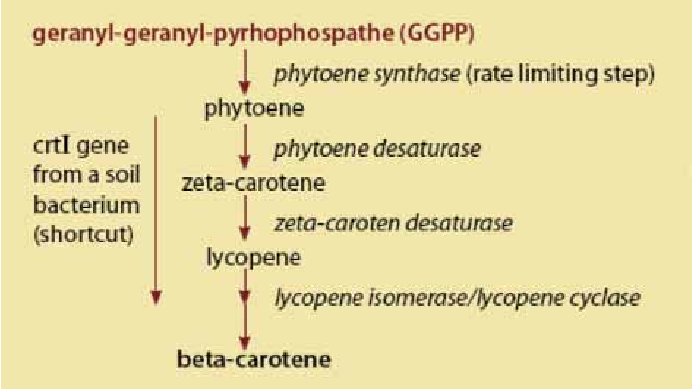


Fig. 4. The provitamin A pathway with rate-limiting steps and genes required to engineer the synthesis of provitamin A in rice endosperm. The plant uses four enzymes to convert the precursor (GGPP) to provitamin A (beta-carotene). These genes are active in all green tissues of the rice plant, but are not active in the seed storage tissue, the endosperm. Three of the missing enzymes (produced by an active gene) can be replaced by one bacterial gene, providing the possibility to achieve the goal with the introduction of two genes only. Proof-of-concept was gained with the four plant genes. To date all novel Golden Rice varieties are produced by the two-gene system.

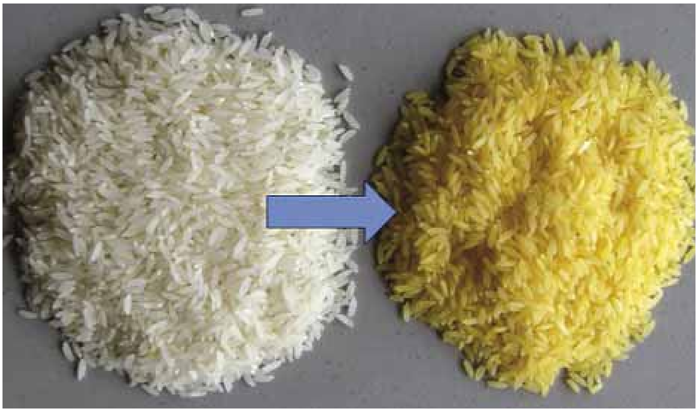


Fig. 5. 'Golden Rice' is a novel rice variety which is based on genetic engineering. It contains sufficient amounts of provitamin A to prevent vitamin A-malnutrition of rice-dependent poor societies, if consumed instead of ordinary rice. The 'directed' evolution of provitamin A-rice was planned, in response to the need, and executed on the basis of the molecular knowledge about the biosynthetic pathway and state-of-the-art gene transfer technology. Such provitamin A-rice never developed during natural evolution, and there is no 'incentive' for evolution to develop such a plant, because there is no selective advantage in any ecosystem for this trait.

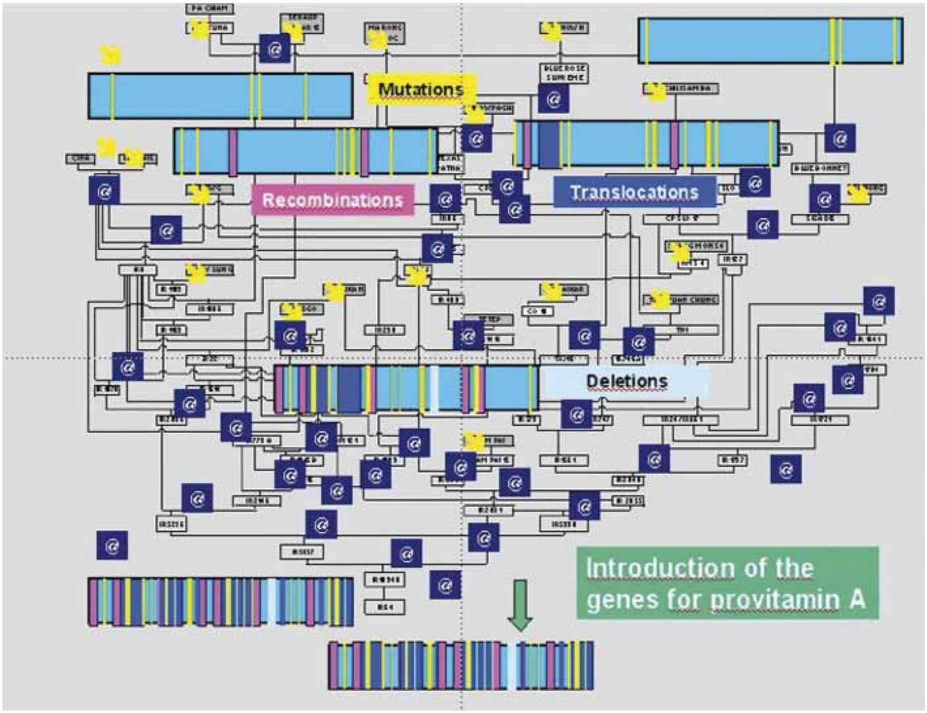


Fig. 6. A relatively minute additional alteration of the genome of the precisely studied genes for the biochemical pathway for provitamin A does not lead to a novel quality of insecurity or unpredictability. But the novel phenotype has the potential to rescue millions of children from blindness and death.

Fig. 7. Peter Breughel the Elder, *The Harvest*, 1565.