<u>Appendix</u>
Plants and their breeding – at the cutting-edge from Stone Age to Green Era

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1. Plants, pests and nutrition

Most wild plants have ***not "intended" themselves to be eaten by man*** (with the exception of certain fruits and berries, whose seeds we are valid to spread). On the contrary, human beings are (by full reason) "considered" as pests by the plant kingdom.

Consequently, <u>plants defend themselves</u> against us with a vast array of means, particularly with toxic metabolites. More than 200 000 small-molecular "secondary metabolites" are already known in plant kingdom, and a majority of these have functions in defence.

2. Developing cultivated plants

First cultivated plants (11 000 years ago) were still quite near to their wild progenitors in characteristics. ***Much work has been required***, and done, to make plants better suited for human cultivation and uses.

In many crucial staple crops the <u>yields have increased 10- to 30-fold</u> during their millennia of cultivation and breeding. (About half of the increase owes to breeding).

Furthermore, many <u>harmful or poisonous wild plants</u> have been transformed to <u>healthy food and nutritious feed</u> during plant breeding.

Wild cassavas were deadly cyanogenic and wild potatoes highly toxic. Rapeseed oil damaged people with erucic acid for 4 500 years. Rapeseed oil was transformed to health-promoting canola oil as late as in the 1960s, by silencing the erucic acid gene in the plant with the help of mutation breeding.

In the evolution of natural and cultivated plants, ***species hybrids and polyploidy*** (genome multiplications) have been very common and much more important than in animal kingdom. For example, cultivated strawberry is a hybrid between European and American species, and its chromosomal constitution is octoploid (it contains the basic chromosome set in 8 replications). And triticale (ryewheat) is a hybrid between altogether four different grass species.

3. Breeding by traditional mass selection (11 000 years)

In mass selection the properties of a plant population are improved by choosing seed from its best plant individuals for sowing the subsequent generation.

Mass selection is the oldest method in breeding, already practised by ”instinctive breeders” in the Stone Age. The method may prove useful still today in novel cultivated species with considerable genetic variation and without a long history of breeding. Whereas in most important staple crops in the world its efficacy has been lost long ago.

Though, selection as such is utilized as a step in all breeding systems, gene technology included, also in the future.

4. Hybridization breeding (~300 years)

Not until plant sexuality was discovered (at the end of 17^{th} century) did the intentional crossing of plants become possible.

In ***traditional hybridization*** the beneficial genotypes of the cross-parents are lost in the progeny.

In ***cross-pollinating plants*** each variety often represents a unique, highly heterozygous genotype, which has been vegetatively multiplied into millions of plants. Such cloned crops constitute e.g. potatoes and many other root plants, bananas, strawberries, currants, most fruit trees, and grapevines.

Such a variety has typically been selected amongst even hundreds of thousands of progeny individuals during many years in field trials. The serendipity can never be repeated. When the unique genotype of the parent is disassembled in hybridization, it is lost forever thereafter. All the king's horses cannot ever build it again in the progeny.

Therefore, popular varieties of apples, strawberries, potatoes etc. cannot be retained and improved in classical breeding but are replaced by unequal new varieties. That means a recurrent loss of huge efforts and achievements (a story far too familiar to the antique loser, king Sisyphos).

Whereas the desired gene can be bred in a plant variety without crossing, provided ***gene technology*** is being used. Thus, the favourable genotype of any popular old variety can be saved practically intact, and the trait in demand is only added to its repertoire.

> Breeding prospects: Burbank potato can be turned resistant to potato late blight, and Royal Gala apples be made immune to bacterial fire-blight disease. Abandoned high-quality banana, Gros Michel, can be returned to deli stores by breeding it to resist devastating fungal diseases.

Though, in ***self-pollinating plants***, e.g. barley and wheat, varieties are often homozygous 'pure lines'. Accordingly, their genotypes can largely be restored *via* further crosses and backcrosses during a score of progeny generations. Much time and effort is required, however, but finally the new gene may often be successfully added to the original genetic constitution of the parental pure-line variety even by traditional means.

Screening of scores or couple of a hundred of experimental plants is usually needed even with ***gene technological*** breeding. Namely, the desired functioning of the gene depends in part on its place of attachment in the chromosome. (Anyway, there exist thousands of positions in plant genome where the gene can function well.)

Consequently, thousand times fewer progeny individuals need to be screened through than if one should rely on classical crosses and random genetic recombination.

Anyway, the historically good success record of plant breeding depends on its established practice that only the selected few best-functioning plants are retained in each step.

5. Traditional wide crosses (> 100 years)

A useful trait (e.g. disease resistance) is traditionally retrieved from a related plant species or primitive plant line into a cultivated variety by hybridization.

In addition to the desired one(s), ***thousands of unknown and unwanted genes*** are transferred into the plant as hitchhikers. In practice, many of the latter ones may turn out to be harmful; for example coding for toxins or allergens or impeding cultivation.

Case: blight-resistant potato

Many of the hitchhikers may be ”diluted” away by dozens of generations of backcrosses with the cultivated variety. However, such a dilution is not reliable but only statistical in character. Hundreds of alien genes may finally remain in the genome, in particular when the desired one is situated near the centromeric region of the chromosome.

In traditional breeding, such swarms of unknown genes cannot in practice be resolved for hiding harmful effects, e.g. allergenic potential. Consequently, no such detailed studies are required or made.

Whereas targeted safety studies can be (and always are) performed for the selected few, well-characterized genes in use when applying ***gene technology*** in breeding. Consequently, the latter technology may often be evaluated to be safer in terms of allergy troubles, as for instance stated by the Union of the German Academies of Science and Humanities in 2004.

Traditional wide crosses also trigger ***chaotic genetic modification*** in the genome.

Jumping genes once calmed down permanently (these may comprise over 40 percent of the genome) are activated and restart jumping in the chromosomes. Hereby swarms of random mutations are generated in the plant.

Wide crosses also reorganize the texture of gene silencing in the chromosomes. Great numbers of plant genes are normally in a permanently silenced state due to methyl groups attached on their DNA sequences. Such "sordini" now become detached, and hidden genes start functioning. Meanwhile the loose methyl groups fix anew and may extinguish essential active genes at random.

Furthermore, the frequency of ***somatic recombination*** is increased. Accordingly, new genetic combinations are produced within plant tissues even in the absence of sexual reproduction.

It is true that all such stochastic processes generate new genetic variation, which is the prerequisite both for natural and man-directed evolution (plant breeding). Regarding cultivated plants developed through hundreds of years of effort, however, such a new variation should absolutely be kept as controllable as possible. Or else we shall start once again from beginning, i.e. invent the wheel anew.

Chaotic genetic phenomena like those can be avoided by picking the desired gene up in the plant alone, in a purified form, i.e. by applying gene technology in plant breeding.

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Fig. 1. **"Karukka"** ("goorrant") is a hybrid between karviainen (gooseberry) and mustaherukka (blackcurrant). Traditionally in plant breeding, all the thousands of genes from two different plant species are at liberty to be combined together without official control. That still holds today. Whereas transferring just a single, carefully studied gene out of this mess in a purified form (applying current and better controllable methods) is strictly constricted with provisions.

6. Classical mutation breeding (60 years)

Natural mutations occur in a plant at a low frequency (say 10^{-6}) as a consequence of DNA damages caused e.g. by errors in cell metabolism, cosmic radiation, natural harmful chemicals, and viruses. Mutations have been a prerequisite for all evolution both in nature and plant breeding.

Novel genetic variation has been generated in traditional plant breeding by increasing mutation rates transiently with the help of e.g. ***gamma or X-ray radiation and chemical treatments***. High doses, killing about 50 percent of the seeds, have been applied for optimal mutation yield.

However, random mutagenesis is both unpredictable and inefficient in genetic improvement. Per each desired change, hundreds of thousands of undesired mutations may be generated in a plant genome. Furthermore, even the finally realized improvements are likely to be far from optimum, because random changes are all too rough and cannot be controlled at all.

With the help of ***gene technology***, the fine structure of a gene (or its controlling sequences) can be adjusted in detail and optimized. The gene can be isolated and its structure modified by established molecular biological methods. Various modified gene forms can then be tested for desired functioning in a laboratory. Finally, a few most promising gene variants can be selected for further studies in a breeding program.

7. Lapses in plant breeding

Few lapses have occurred even during the centuries of traditional, trial-and-error breeding. A couple of potato varieties were found producing too high levels of toxic natural alkaloids (solanine, chaconine) - though not nearly as high ones as typically in wild potatoes. Accordingly, such varieties were withdrawn.

An old zucchini variety was maintained by "do-it-yourself-breeding" in organic cultivation in New Zealand. In 2002, it was attacked heavily by insect pests and produced dangerously high levels of a bitter glycoside cucurbitacin (the plant's natural defence chemical against animal pests). Hence, 16 persons were taken in hospital due to intoxication. Newer varieties of zucchini are no more prone to such an exaggerated self-defence reaction.

A new celery variety, bred for organic cultivation, was recorded to produce too high concentrations of psoralen. That is a natural defence chemical causing sunburns in skin. Due to skin injuries in harvesting personnel the variety was withdrawn immediately.

8. Unintended or unexpected changes

Genes may have unexpected effects in combination. Such ***genetic interactions*** were named 'epistasis' by Gregor Mendel in 1866. Interactions between genes have arisen in important position in the quantitative theory of breeding since, say, 1920–30s.

In ***traditional plant breeding***, beneficial genetic interactions are thoroughly utilized. In particular, these are in a key position when developing so-called 'hybrid' varieties, which are popular all over the world thanks to their vigour, productivity and uniform yield quality. Correspondingly, plant lines expressing unfavourable genetic interactions are routinely discarded from breeding programs.

In a traditional cross, huge numbers of genetic interactions may occur. Whereas when a gene is bred by ***gene technology***, only a tiny fraction (20 000 times less interactions) may occur even in theory.

Actually, an increasing number of recent metabolic studies (***metabolomics***) show that plants bred via gene technology have much fewer unexpected changes than plant varieties bred by traditional means.

In a ***traditional cross***, million times higher load of foreign DNA and 20 000 times more[1] abundantly of unintended and unknown genes are typically introduced in a plant, compared to retrieving the desired gene in the plant in a purified form by using gene technology.

[1] Correction (Oct. 18, 2006/J.T.): that statement "20 000 times more" is a mistake and should in strictly scientific terms be corrected to "infinitely times more" (because by applying gene technology, not a single one unintended or unknown gene is being introduced in the plant; and 20 000 divided by 0 is infinity)

9. Gene flow between flowering plants (270 000 000 years)

Exchange of genes between plants via pollen is an ancient natural phenomenon.

During Agricultural Era (11 000 years) some gene flow has even occurred from cultivated plants to related wild ones. However, the traits bred in cultivated plants are meant to bring benefits for man. Hence, these only very seldom give advantage to the plant but weaken it in the Nature. Thus, as a rule, such traits have not invaded natural populations but are efficiently screened away by natural selection.

Cultivated plant varieties are no new species but correspond to their parental cultivars very closely in all issues except their specific freshly-bred traits.

On the contrary, a (low) percentage of ***alien plant species*** may be able of establishing permanent populations or even invading Nature in the receiving country. Even so, thousands of alien plant species have traditionally been freely introduced in gardens all over Europe.

10. Biologically valid legislation

1) According to a broad scientific consensus within the plant sciences community, the legislation governing applied genetics products should be based on the resulting trait and not on the method(s) of breeding used in its development.

That principle was already stated by the European Association for Plant Breeding Research (EUCARPIA) in 1989.

In Canada, legislation is based on such biologically valid concept ("Plants with Novel Traits"). For example herbicide tolerance – when it is a new trait in a crop plant – triggers the legal requirements irrespective of whether the trait was bred using old or contemporary techniques. In EU, on the contrary, the imidazolinone-tolerant canola variety, bred by traditional means in Canada, does not trigger our (method-based) GM legislation.

2) Silencing native harmful genes (e.g. antinutritional ones) in crop plants is ecologically safe and should be exempted from the demand of environmental risk assessment (ERA) in EU regulations (by refocusing their scope accordingly).

Nature has already tested silencing any such gene in a plant over thousands or million times each year, for thousands or millions of years – without evolutionary success. Namely, the changes that remove inherent (often defence) capabilities do not enhance but impair the plant's fitness and competing capacity in the Nature.

3) Enhancing the nutritional composition of a crop for food or feed uses should be exempted from ERA requirements, because the change impairs the crop's fitness and invasive potential in the Nature. Such changed plants are less likely to cause harmful ecological impacts than ordinary plant lines from traditional breeding programs.

4) When desired genes are acquired in a cultivated plant from other taxons or from toxic or wild plant materials, the transfer of purified gene forms with the help of gene technology is safer than old breeding methods and should be preferred. GM legislation should be amended accordingly.

◘ **More detailed information** on **technical and biological topics in breeding** is available in a report prepared by scientific experts in consensus:
"Enabling the coexistence of genetically modified crops and conventional and organic farming in Finland"
http://wwwb.mmm.fi/julkaisut/tyoryhmamuistiot/2005/Trm2005_9a.pdf
[*Gene technology and GM crops:* p. 15–20 & 76–88; *List of references:* p. 62–75.
Please note that the draft translation still contains a few lapses in wording]

◘ **This Appendix** (http://geenit.fi/EP101006App.pdf) was prepared to accompany the presentation "New and emerging applications in biotechnology" (http://geenit.fi/EP101006.pdf) in European Parliament Oct. 10, 2006.