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Plant biotechnology solutions to global questions

Lecture: http://geenit.fi/JAL504jt2013.pdf

Reading: http://geenit.fi/E/Readingjt2013.pdf

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Plant biotechnology solutions to global questions
J.Tammisola Sep 11, 2013

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What every life scientist should get to know in the current 'Era of disinformation'? 1

The flourishing science and industry of plant breeding has been next to destroyed in two decades in Europe. Why in the world, and how?

- Leading GMO opponent Mark Lynas opens up some inside overtones of the offensive against modern life sciences. It has been rolled with unparallel success in media and parliaments for two decades by wealthy 'environmental' lay organizations, 'green' parties and guerillas in the dark; as well as a few new-age religions (Antroposophy, founded by Steiner, and Maharishi-cult or Yogic flyers or Natural Law Parties).
  - "These fears spread like wildfire, and within a few years GM was essentially banned in Europe... Worries were exported by NGOs like Greenpeace and Friends of the Earth to Africa, India and the rest of Asia, where GM is still banned today."
  - "This was the most successful campaign I have ever been involved with. This was also explicitly an anti-science movement."

What every life scientist should get to know: – Seralini study was retracted 2

- A long row of fakes and lousy or "ideologically" forged studies have been circulated by activists over the years
  - aimed at scaremongering people with whatsoever imaginary hazards of GMOs
- The *present media favorite* is the one claiming that GM maize causes cancer, published in 2012 by Eric Seralini, an established* anti-GMO activist
- Though, that hyped study was recently retracted by the science journal itself...
  - because its claims are "not supported by its own study data", as judged by the Science Community in a broad consensus all over the world (see e.g. EFSA press release and final review).

*See Seralini's previous attempt in 2007, ordered by Greenpeace *via* CRII-GEN, another anti-GMO campaign organization: Published results of other researchers were published anew in his name (☺); an odd squiggle in the modern history of science – and certainly not a honour to the magazine in question. The original results were calculated anew with inappropriate statistical methods (but chosen to reach conclusions "in demand").

Crop plants have been – and must be – altered

- 11 000 years ago the start of agriculture and plant breeding enabled the genesis of modern human civilizations
- Vital staple crops were not donated ready and waiting for us
  - ...but were conquered by hard work through millennia
- Natural plants are adapted for their own and not human benefits
- Plants defend themselves against pests (including man) with a multitude of chemical weapons
- Human-directed evolution (plant breeding) has changed crop plants to suit human needs better
- Yields have increased by orders of magnitude (often 10–30-fold)
- Toxic and harmful chemicals have decreased in food crops
- Nutritional contents (e.g. essential amino and fatty acids) have been adjusted towards human requirements
  - ...though great improvements are still necessary

Art of breeding

- Great improvements still need be done in many old traits
  - ...and novel traits are necessary in the changing world
- Art is artificial beauty
  - ...and plant breeding was traditionally praised as classic art by the breeders themselves
- Recent breakthroughs in molecular biology and genetic understanding have
  - *evolved* plant genetics, physiology and breeding to true sciences
  - *improved* both precision (100–100 000-fold) and predictability (thus safety as well), efficiency (often 30-fold), and application potentials of plant breeding
  - *lowered* the cost of breeding new crop varieties a lot (apart from undue costs of approval owing to odd legislation which contrary to science is based on the breeding methods used and not the traits being bred in the plant)
- Consequently, hundreds of biological inventions now lay (growing dusty) on university shelves
- …waiting for financial and social support
  - to push their way through the obstacles of GM and product legislation (to obtain permissions for their cultivation and use)
- ...to be finalized and made good use of for the prosperity of man and nature

Novel prospects and challenges

- **Quality, Nutrition, Health, Environment, and Bio-economy**
  – five core drivers in the rapidly changing world
- For the past 11 000 years, plant breeding has generated about one half of the progress in the productivity of agriculture
  – other half is owing to improved cultivation technologies
- ...but now it shall meet even bigger challenges
- Climatic change alone (direction disregarded...) would call plant scientists for a herculean task:
  - ✓ Update the existing thousands of key plant varieties to be suited to the changed conditions
  - ✓ Breed a new generation of staple crops provided with crucial new traits, such as
    – radically enhanced nutritious qualities (for better growth and health with even less feed and reduced growth area)
    – drought, heat, frost, salt or flooding tolerance
    – much more solid resistance to possibly ever more prolific plant pests and diseases
- Such updatings are vital just for retaining our current yield levels and efficiency in cultivation
  - ✓ ...but really improving food security would call for true (no-nonsense) attitudes towards life sciences

EASAC (2013): Second generation of GM plants is being bred with unparalleled purity, safety, precision and knowledge

- “**Europe should rethink its stance on GM crops**. The objective must be to **regulate the product and not the technology that produces it**.”
- In last years, farming efficiency has ceased to be a priority in EU. And so, EU countries now grow less than half the food and animal feed they are consuming
  - Hence, Europe has slid to the world’s largest importer of agricultural commodities
- Due to its ’magic’ campaigns and anti-science policy Europe lost the substantial benefits of the first generation GM varieties altogether – even if the technology itself was just originated from European universities
  - As one inevitable by-product, our flourishing **plant breeding research and industry was lost** almost in total: university chairs are vanishing and all competitive breeding companies have moved to other continents
- In face of the great environmental and economic challenges, our stagnant continent **cannot afford losing the second generation** of GM plants as well
- ...warns our highest scientific body, the **Council of European Science Academies**, in its thorough report ”**Planting the future: opportunities and challenges for using crop genetic improvement technologies for sustainable agriculture**” (EASAC 2013: full report, short, and lay [non-technical] version)

Vital improvements are urgently needed in drought, heat, frost, salt and flooding tolerance of key crops

- **Significant advances are going on both in finding out their plant physiological mechanisms and in breeding better tolerant crops using modern biotechnology**
- Though, due to the strict time limits, these important tolerance traits must be for the most part skipped in the present lesson

- *Introduction:*
  Tammisola J (2010). Review: Towards much more efficient biofuel crops – can sugarcane pave the way? GM Crops 2010; 1:181–198
  http://www.landesbioscience.com/journals/gmcrops/02TammisolaGMC1-4.pdf
  or its manuscript equipped with hyperlinks:
  http://geenit.fi/E/GmCrops10.pdf
- *Up-to-date perspectives:*
  Schroeder et al. (2013). Using membrane transporters to improve crops for sustainable food production. Nature 2 May 2013: 60–66
  http://dx.doi.org/10.1038/nature11909

New modification tools offer unprecedented precision* in editing native genes in plants

- E.g., enzymes such as TALENs (transcription activator-like effector nucleases), and ZFNs (zinc-finger nucleases)
- ...can cut plant DNA at specific points chosen by the breeder
- By controlling how this break is repaired, it is possible to introduce exact
  - mutations
  - single-nucleotide changes*, or even
  - whole genes at precise sites
- The new gene can be put in a spot in the genome where its expression is optimal
- Hence, plant’s genome is no more disrupted in undesirable ways
- ...as was common in traditional breeding and even classic genetic modification
- At least 6 to 8 such high-precision new methods of DNA modification are already in use in plant breeding

(Cressey 2013, Nature 02 May 2013: 27–29)

*New precision modification is at least 10 billion-fold as accurate as conventional mutation breeding

Genome editing:
Membrane transporters & sustainable food production

Transport proteins embedded within plant membranes are key targets for improving the efficiency of

- **uptake and use of nutrients and water , and**
- **movement of sucrose to where it is needed**

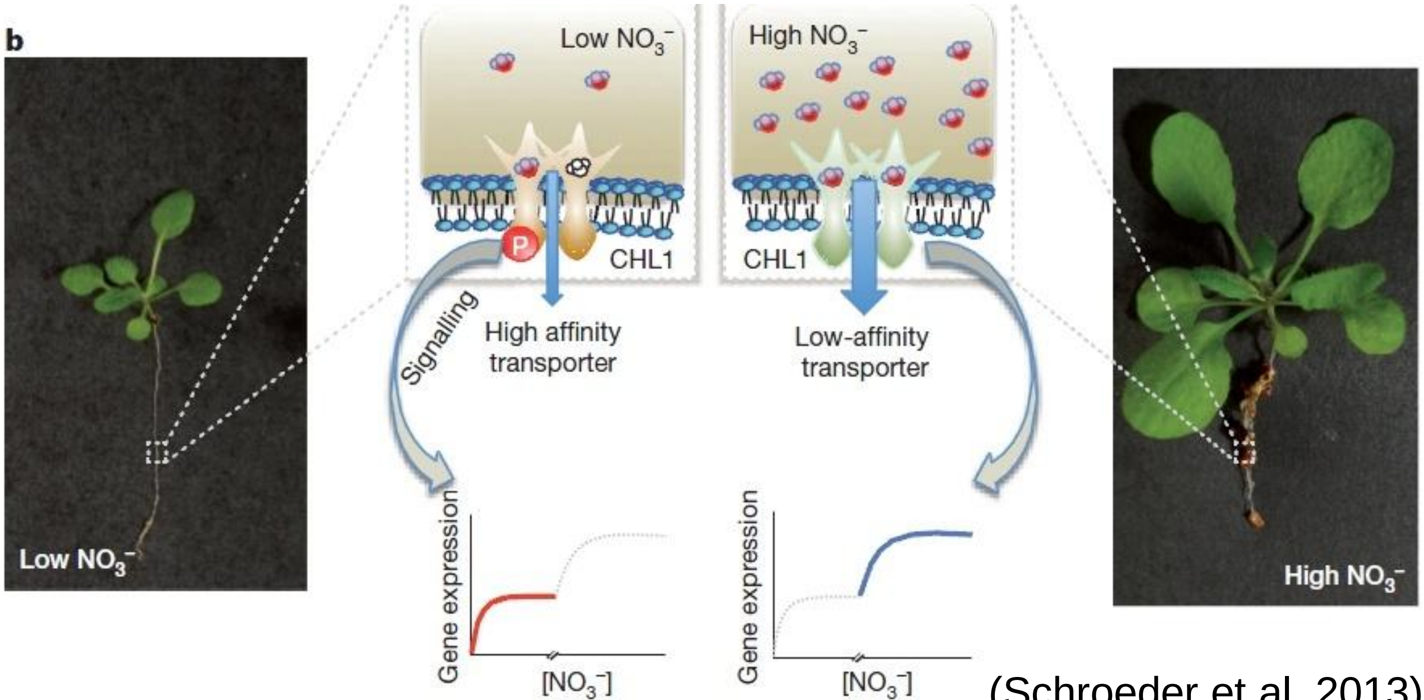
- **...and tolerance to adverse environments such as saline or acid soils**

Specialized plant membrane transporters can be used to

- **Enhance yields of staple crops**
- **Increase nutrient content, and**
- **Increase resistance to key stresses, including**
  - **salinity**
  - **pathogens, and**
  - **aluminium toxicity**
- **...which in turn could expand available arable land**

(Schroeder et al, Nature 02 May 2013)

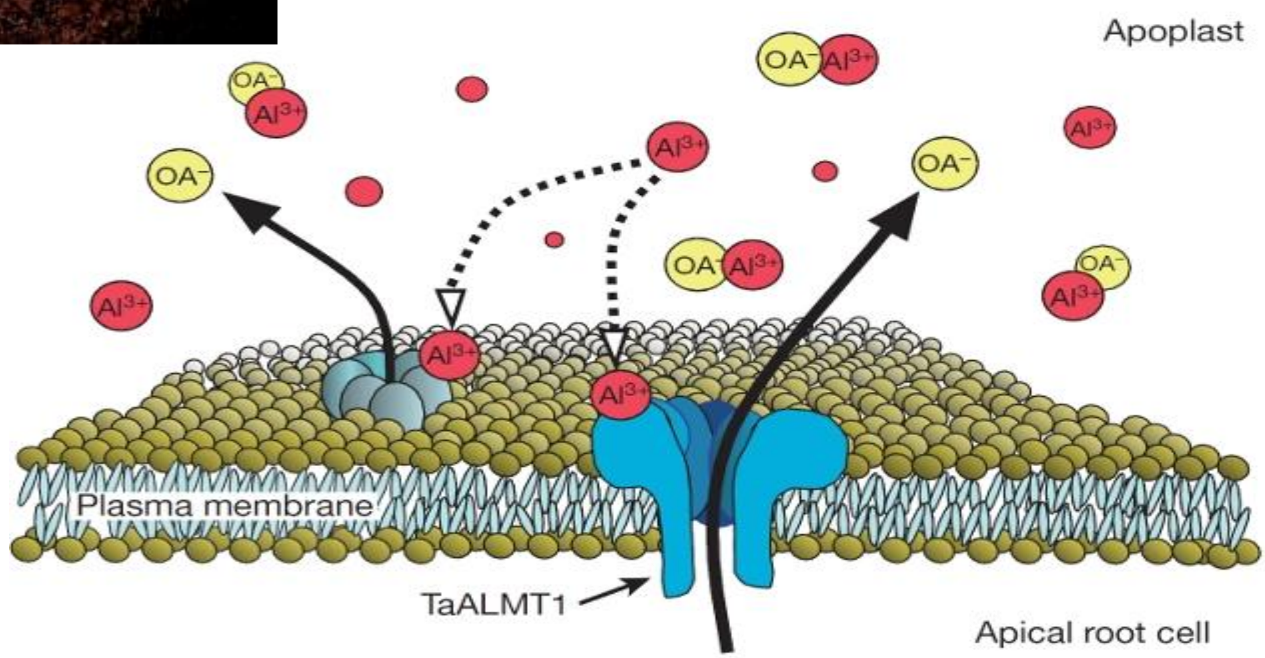
Nitrate transporters sense and regulate nitrate uptake



(Schroeder et al, 2013)

- At low nitrate, CHL1 is phosphorylated at a specific amino acid, T101,
  - converting it to a high-affinity nitrate uptake transporter,
  - enabling nitrate accumulation at limiting soil nitrate concentrations.
- At high nitrate, the T101 amino acid is not phosphorylated, and
  - CHL1 functions as a low-affinity transporter.
- Potential tools for engineering crops with tailored N uptake activity, N metabolism and improved root growth for enhanced nitrogen-use efficiency.

Aluminium-tolerance into barley with an Al^{3+}-tolerance transporter gene from wheat (*TaALMT1*)



(Schroeder et al, 2013)

- TaALMT1 anion channel within the plasma membrane of apical root cells is activated by Al^{3+} so that malate molecules (OA^{-}) flux out.
- In the apoplast, external to the cytoplasm, malate molecules bind Al^{3+} to protect cells from aluminium toxicity at the root apex.

Golden Rice: Sufficiently of vitamin A from rice-based diets for billions of people

- See JAL504 Sep. 25, 2013:

”**Success stories in plant breeding**”

by Peter Tigerstedt and Teemu Teeri

Cassava – food security for resource poor subsistence farmers 1.

(Sayre et al. 2011)

- Cassava (*Manihot esculenta*) was introduced in Africa from Brazil 500 years ago
- Its starchy root crop is now the staple source of calories for 800 million people in the Third World
  - including more than 250 million Africans , especially in Sub-Saharan Africa

Cassava – food security for resource poor subsistence farmers 2.

(Sayre et al. 2011)

- Its agronomic traits suit for a food security crop
  - Cassava needs limited human input
  - It can be reliably grown in marginal conditions, e.g. in poor soils, and it is unusually drought tolerant
  - Harvest times are flexible: roots can be dug up all through the year (undisturbed in the soil they can be stored up to 3 years)
  - Its cyanogenic glycosides (linamarin and acetone cyanohydrin) reduce crop losses due to generalized herbivores

Cassava varieties are propagated [unaltered] from stem cuttings

Cassava – food security for resource poor subsistence farmers 3.

(Sayre et al. 2011)

- Though, cassava has a number of **major disadvantages**
- The plant is susceptible to virus diseases, especially CMD (Cassava Mosaic Disease)
  - caused by a geminivirus endemic to all the cassava-growing regions of Africa
  - impairing cassava yields by 30–40 % in affected areas
- Shelf life of the roots is very short:
  within 72 h after harvesting, the storage roots deteriorate and are inedible, which
  - causes high storage losses
  - severely reduces the possibilities of farmers to sell their crop in the markets
- Roots are deadly poisonous so that much energy and work is lost in extensive food processing
  - to avoid chronic (goiter, tropical ataxic neurophathy) or acute (permanent paralysis, death) intoxication disorders
- Their nutritional value is low except calories

Traditional cassava is nutritionally poor food

- Cassava roots are a sufficient source of energy (in the form of starch)
- ...but are poor in other nutrients such as protein, vitamins and key minerals (iron and zinc)

Vitamin A Fortified Cassava   Traditional Cassava

Traditional cassava is deprived of vitamin A. Accordingly, vitamin A deficiency is a big cause of deaths in the regions with cassava-based diets

- Cyanogens (linamarin and acetone cyanohydrin) accumulated in the roots must be removed by a thorough processing during 3–6 days
  - traditionally by grating, fermentation, cooking and drying
- ...which may reduce the contents of essential nutrients even further

Micronutrient deficiency disorders increase mortality in developing countries

Annual deaths due to micronutrient deficiency disorders
(Source: Caulfield et al. 2006)

Region and its population size [10^6]		**Vitamin A deficiency**	**Iron deficiency Anemia**	**Zinc deficiency**
East Asia and the Pacific	1,823	11,000	18,000	15,000
Eastern Europe and Central Asia	475	0	3,000	4,000
Latin America and the Caribbean	524	6,000	10,000	15,000
Middle East and North Africa	301	70,000	10,000	94,000
South Asia	1,378	157,000	66,000	252,000
Sub-Saharan Africa	674	383,000	21,000	400,000
High-income Countries	957	0	6,000	0
Total	**6,132**	**627,000**	**134,000**	**780,000**

- A typical adult-sized cassava meal (500 g) can provide adequate calories but it meets only 30% of the minimum daily requirement (MDR) for protein, iron and zinc and 10% of the daily provitamin A (β-carotene) requirement
- Cassava also has the lowest protein-to-carbohydrate ratio of the world's 10 major crops.(Sayre et al. 2011)

BioCassava Plus (BC+, BCP): One daily serving of biocassava should provide complete nutrition. 1

- BC Plus is an innovative research project that aims to reduce micronutrient malnutrition by increasing the nutritional value of cassava
  - Integrated team of public sector scientists and nine institutions from Africa, Asia, Europe, Latin America, and North America
  - Focused on sub-Saharan Africa, especially Nigeria and Kenya, where cassava is a major staple food and other nutritious food is scarce, unavailable or too expensive
  - The program has been supported with important grants by Bill & Melinda Gates Foundation
    - ”Activists” propagate their ”Stop Gates” –campaign...
- Objectives (in part):
  - Create and field test a transgenic variety of TME7 cassava in Nigeria enhanced with β-carotene such that it contains 40 μg -carotene/g dry weight and iron such that it contains 40 μg iron/ g dry weight.
  - Create and field test a transgenic cassava variety 'Serere' in Kenya that is resistant to cassava mosaic disease and cassava brown streak disease, enhanced with β-carotene, iron and protein.
  - Prepare the farmers, processors and public for acceptance and adoption of Biocassava Plus varieties
  - Carry through the studies and applications needed for gaining permissions for their cultivation and use

BioCassava Plus (BC+, BCP): One daily serving of biocassava should provide complete nutrition. 2

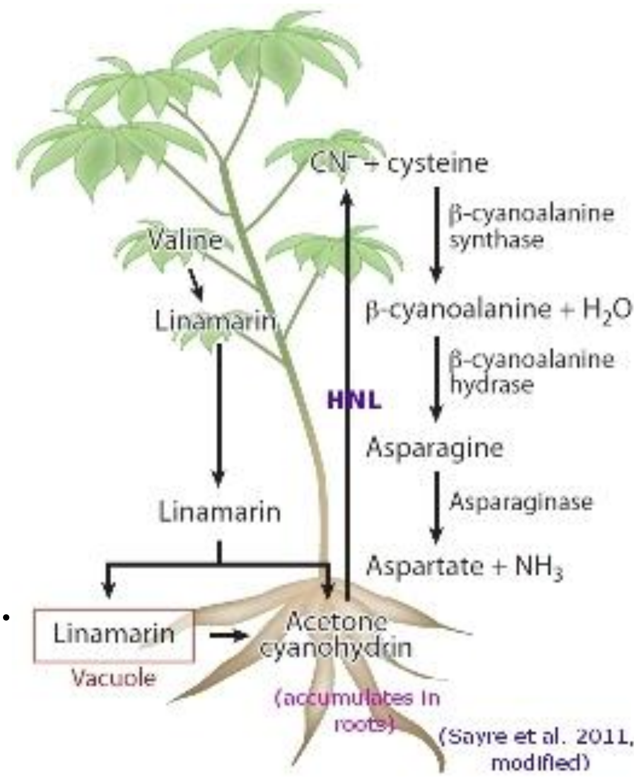
- Phase I (2006–2010) of the program met or exceeded all its targets to date (Sayre et al. 2011). Applying GM the team has developed cassava plants that have
  - 30 times as much beta-carotene
  - four times as much iron
  - four times as much protein as traditional cassava
  - improved virus resistance attained by expressing a protein or short interfering RNA sequences with an inhibitory effect on the virus in the plant.
  - Furthermore, cyanogen content in the roots has been reduced by 80 %, and
  - shelf life of cassava roots has been extended from three days to 3–4 weeks.
- Field tests have been started in Nigeria (2009) and Kenya (2010)
- Such improvements are often untenably slow or even impossible using traditional breeding methods
  - because breeding progress is often hampered or stopped altogether due to narrow or missing genetic variation with regard to the trait in the breeding population

Enhancing protein content in cassava roots 1. (Sayre et al. 2011)

- Cassava roots contain on average 1–2% protein by dry weight, substantially less than maize
- Various GM strategies were tried in enhancing it

1. Resources were redirected from cyanogens (linamarin) to protein synthesis in the roots

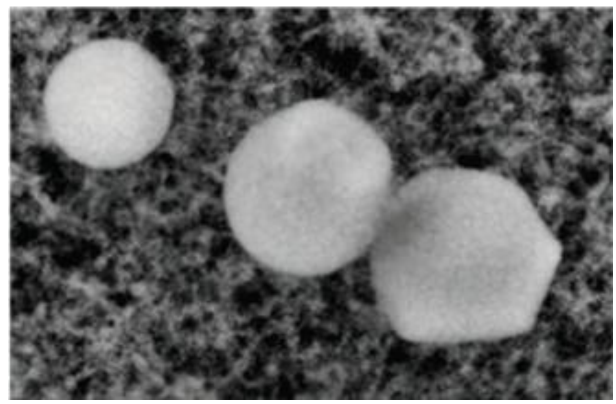
- Linamarin is synthesized in leaves and partly transported to roots, where it is either stored in the vacuole or metabolized to provide reduced N for amino acid synthesis.
- Acetone cyanohydrin is built up and accumulated in the root. In digestion it is spontaneously broken down, releasing cyanide.



- Cassava hydroxynitrile lyase (**HNL**) catalyzes the conversion of acetone cyanohydrin to cyanide in the leaves.
- When its gene was equipped with root-specific patatin promoter (from potato), it started functioning also in the roots
- ...and improved their protein content **threefold**
  - As a bonus, root linamarin was reduced by 80 %. In addition, cyanide can now be removed 50 times faster in processing.

Enhancing protein content in cassava roots 2. (Sayre et al. 2011)

- **2. Roots were modified to express chimeric storage proteins rich in essential amino acids**
- The fusion proteins were designed to form protein bodies which would accu-mulate in the endoplasmic reticulum.



Purified zeolin protein bodies (3–7 µm) isolated from GM cassava storage roots.

- Zeolin is a fusion protein of phaseolin from *Phaseolus vulgaris* and gamma zein from *Zea mays*, expressed under control of the root-specific patatin promoter (from potato)
- Total protein content was increased **fourfold** in the roots of GM cassava producing zeolin
  - whereas their linamarin content was reduced by 55 %
- Though, bean proteins may give rise to food aller-gies more readily than storage proteins on average
- Hence, for the next steps, phaseolin component is replaced with other proteins in the fusion proteins being tested in the program

Cassava root shelf life was extended to 3–4 weeks by GM

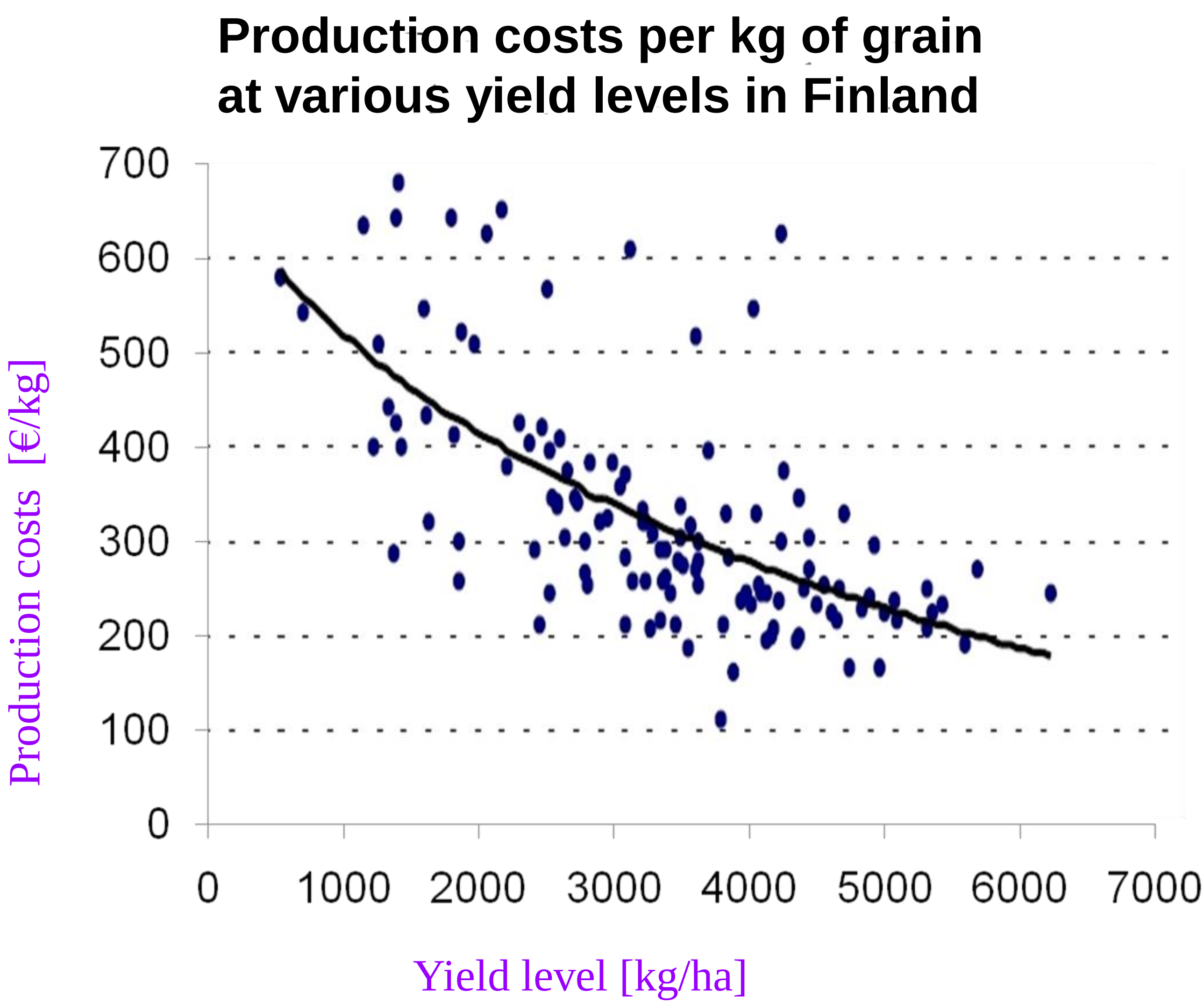
(Sayre et al. 2011)

- Cassava roots deteriorate unpalatable and unmarketable within 24–72 h after harvest
  - by an active process involving gene expression and protein synthesis
- The start of the postharvest physiological deterioration (PPD) is signaled by a rapid oxidative burst accumulating reactive oxygen species (ROS) in the roots within 15 min of harvest
  - predominantly superoxide and hydrogen peroxide
- In cassava roots, cyanide blocks cytochrome C oxidase, resulting in the generation of ROS
- Overexpression of a cyanide-insensitive alternative oxidase (*Arabidopsis* AOX) in GM cassava roots resulted in substantially reduced ROS accumulation
- ...and delayed the onset of PPD by **3 weeks**

  Roots od traditional cassava (left row) are fully rotten whereas the best GM cassava lines are still healthy in day 21 after harvest

- Another strategy was to overaccumulate antioxidants. Indeed , the shelf life of GM plants with elevated β-carotene (40 ppm) content was extended to **4 weeks**.

Productivity is the major determinant of production costs in crop husbandry

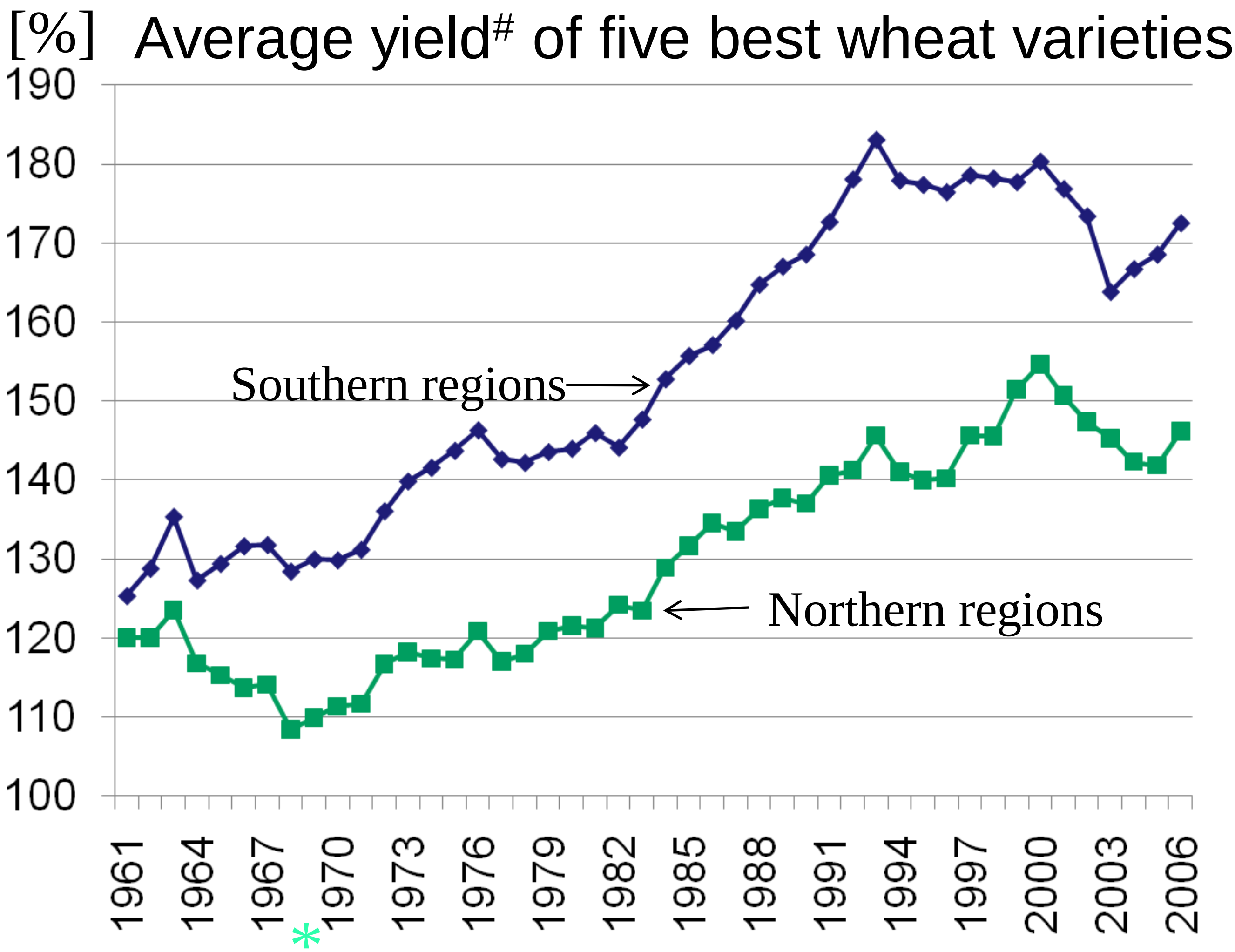


- At low yield levels the production costs per kg of grain increase multifold

Riepponen L (2003). Maa- ja elintarviketalous 19, MTT, 32 p.

http://www.mtt.fi/met/pdf/met19.pdf

Retarding progress with dated breeding: Genetic gains in yield levels of winter wheat are being halted since late 1980's



Expressed in % relative to the yield of the check variety Kharkof

* Introduction of the Green Revolution varieties in wheat

Relative grain yield vs. year, for the five top yielding entries in official comparative nursery trials in the Great Plains of North America, 1959–2008.
Figure points stand for five-year moving averages.
Graybosch & Peterson, *Crop Sci*. 2010; 50:1882–1890

- Further improvement in the genetic potential for grain yield awaits some new technological or biological advance

Harnessing bio-economy calls for new achievements in biological efficiency

- Achieving high productivity is the key question for reaching true sustainability in bio-economy
  - ...biofuels included
- Or else huge impoverishing automata are being constructed for ruining our civilization
  - ...duly comparably with the perpetual-motion economical machine in Stalin's agriculture which sucked Soviet national economy dry during a few decades:
    - ✓ E.g., nourishing swine and cows with bread instead of feed was made apparently ”profitable” (but surely not sustainable)
- Biotechnological solutions beating old technologies in yield, energy use and costs
- ...must be created during the upcoming few years
  - ✓ ...which means troubled waters for EU, mixed-up with its occult movements and firmly established anti-science policies
    - ...petrified in its biologically untenable GM legislation
- E.g. oilseed rape should be bred resistant to clubroot, so it could be grown in successive years
  - ...increasing its potential cultivation area fivefold in EU

Inefficient biofuel plants compromize food security?

- Regarding transport biofuels, just bioethanol from tropical *sugarcane* is reasonable in terms of carbon balance and economy (IEA 2007)
  - sugarcane is grown on more than 20 million hectares, one third of that in Brazil
- Maize yields very little ethanol per ha
- Poor efficiency in biofuel production deprives food production of its field area
  - ...or extra land for cultivation must be cleared from the Nature in a large scale
- Pursuing ecological and economic sustainability in biofuels, the productivity and eco-efficiency of biofuel crops must be greatly enhanced (EPSO 2007, Tammisola 2010)
  - in a short time, and consequently
  - ...based on modern plant breeding, especially genetic modification

Tropical sugarcane is far and away the most eco-efficient crop plant in ethanol production

Sugarcane fields in Luxor, Egypt, Oct. 15, 2010. © J.Tammisola

In Egypt, sugarcanes exceed 2 m in height, whereas in Brazil they grow doubly higher

To no surprise: the core of the stem tastes cane sugar ... The liquid pressed from the canes contains 17–22 % sucrose

- Tammisola J. Review: Towards much more efficient biofuel crops – can sugarcane pave the way? GM Crops 2010; 1:181–198 http://www.landesbioscience.com/journals/gmcrops/02TammisolaGMC1-4.pdf

Why is it hard to breed sugarcane by traditional means?

- Cultivated sugarcanes are
  - Highly polyploid (ploidy levels 5x–14x) and even aneuploid plants (i.e. contain extra or missing copies of chromosomes)
  - Species hybrids: *Saccharum officinarum* (2n=80) *x S. spontaneum* (2n=40–128)
  - Highly heterozygotic ”jackpot hits”, cloned to millions of copies for cultivation
  - ...because their superior genotype would be lost in sexual reproduction (especially crosses)
  - Slow to grow from seed to maturity
  - ...and mostly almost sterile in practice
- Accordingly, improving an elite variety further with crosses translates to a fairly desperate ”Sisyphos”-affair statistically
- Thus e.g. sugar content has not improved much at all in 40 years (Jackson 2005)
  - ...despite heritability occurring in the trait

Too little progress in sugar content

- Sugar content is influenced by a multitude of genes (each one with a small effect as a rule)
- High-sugar genes (alleles) derive from *S. officinarum*
- Enriching such profitable genes together in a single superior genotype is very hard work by old means in polyploid hybrids
  - …because each basic chromosome type may occur in up to 14 (related) copies in the cell
  - ...and because all the other important traits shall also be kept unimpaired in the process
- Crosses break down the elite genotypes
  - …to a (”creative”) statistical chaos
  - ...and at the same time, arrays of poor alleles (once already screened out with hard work) make re-entry to the breeding lines once again

Sugar content was doubled with one step of genetic modification in sugarcane (see Tammisola 2010)

- By applying GM to an elite cane variety, its sugar content could be doubled (Wu & Birch 2007, Birch 2006)
  - ...without compromising its unique genotype
- A bacterial gene for sucrose isomerase enzyme was inserted in the plant
- In addition to normal amounts of sucrose, GM sugarcane yields similar amounts of its isomeric form (isomaltulose)
- Isomaltulose is a health-promoting polysaccaride
  - produced for funtional foods by bacterial fermentation
  - ...and also suited for being fermented to alcohol
- That sugar isomer is not utilized by the plant itself, and hence it is accumulated without loss in sugarcane cells
  - ...where it was channelled to find its way into vacuoles
- Field trials are going on in Australia (OGTR 2005 a, b)

Sugarcane is being bred to split its cellulose into sugars itself?

- Sugarcane produces more than 200 metric tons biomass per ha
- The plant is being bred to split the cellulose of its cell walls into fermentable sugars itself
  - without expensive pretreatments or purchasing costly enzyme preparations
  - ...so that exhausted pulp can be used for cellulosic ethanol production at a reasonable cost
- Sugarcane is modified genetically with genes coding for enzymes needed in spitting cellulose into sugars that can be frermented into alcohol
  - costly enzymes are produced gratis in plant cells
  - delivered from inside the cell the cellulolytic enzymes have better access and efficiency, so that there is less need for expensive pretretments
  - the genes are turned on not earlier than 2–3 days before harvest so that the cellulolytic enzymes do not harm plant growth
- Self-splitting sugarcane is being developed by a research coalition between Leaf Energy (Aus.) and Syngenta http://www.leafenergy.com.au/other.php

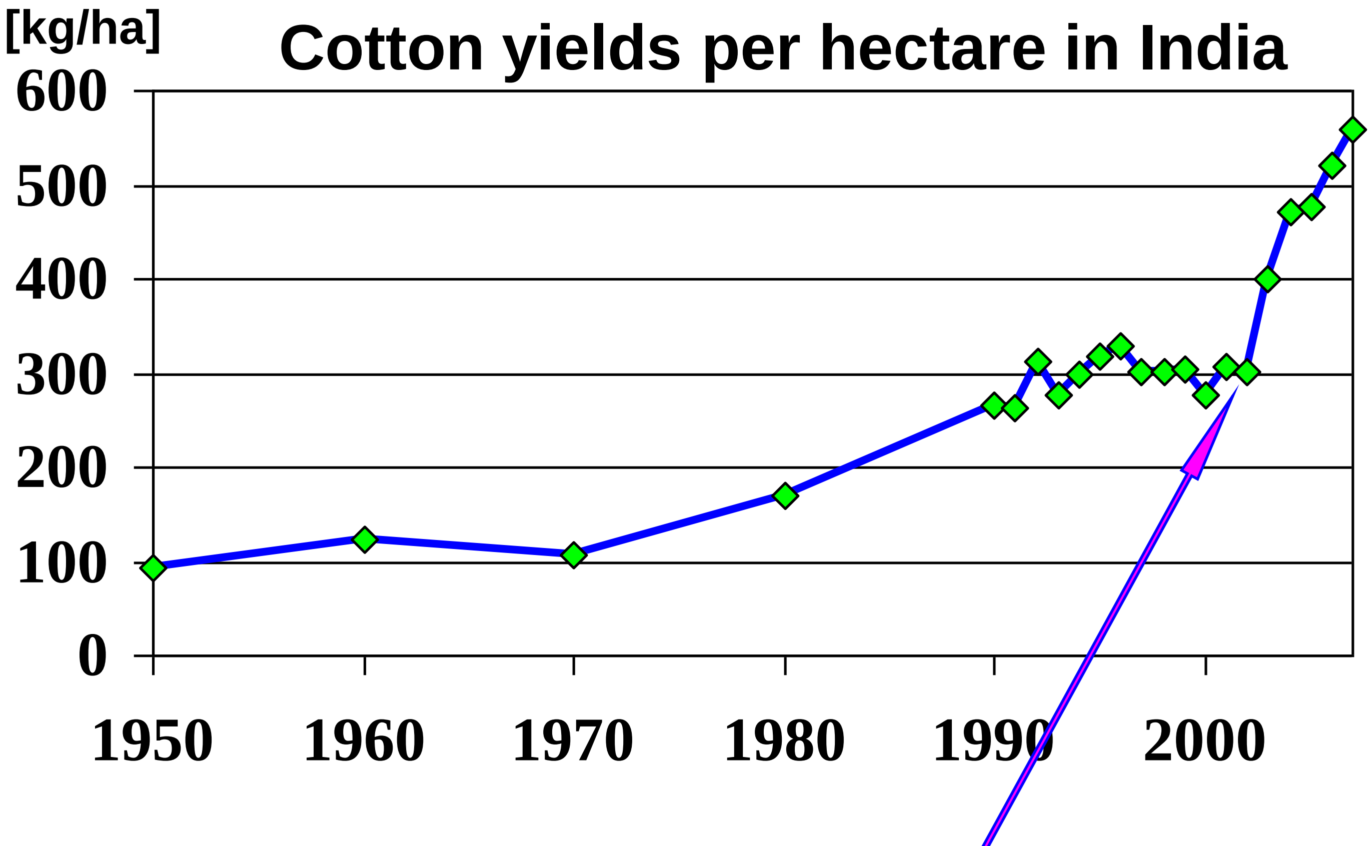
Cell wall lignin in sugarcane is being GM to a better degrading constitution in Brazil

- Cell wall lignin is the hardest barrier for the cellulolytic enzymes
  - …because the combined cover of lignin and hemicellulose prohibits the access of cellulolytic enzymes into cellulose fibers
- The pulp remaining after pressing sugar out of the canes consists of:
  20–25 % lignin,
  23–30 % hemicellulose, and
  45–50 % cellulose
- Lignin is tough to break down
  - …especially recalcitrant type of lignin (guaiacyl)
  - another type of lignin (syringyl) can be degraded much more redily
- Hence, lignin in sugarcane cell walls is being GM to a better-degrading type consisting almost exclusively of syringyl (Allelyx SA, Brazil)

- Tammisola J (2010). GM Crops 1:181–198. http://www.landesbioscience.com/journals/gmcrops/02TammisolaGMC1-4.pdf

Are we occupying natural resources for the production of cotton or invasive bollworms?

Bollworm-resistant GM cotton rescued Indian cotton livelihoods



- Bt-cotton cultivation in the country started in 2002
  - Varieties resistant to cotton bollworm are now being cultivated in over 90 % of Indian cotton acreage
  - Yields per hectare have risen 80 % during but 6 years (Official Cotton Statistics)
- Inexpensive Bt-cotton varieties suited for non-irrigation areas have been relased by public research sector in India
- Water shortages can be met with cotton modified for drought-resistance (under development)

Bollworm-resistant GM cotton rescued Indian cotton farmers

A steady state of tragedy

Contrary to popular myth (cf. Vandana Shiva) , the introduction in 2002 of *Bt* cotton is ***not*** associated with a rise in suicide rates among Indian farmers

(*Nature,* May 2, 2013)

- Bt-cotton cultivation has not increased but in fact slightly reduced farmer suicides in India as is concluded in an independent study review (IFPRI 2008)
  - ...by diminishing their risk to crop failures
  - ...and by improving their cotton yields, income and occupational safety & health (Tammisola 2006)

Edible cottonseed – high-quality protein to feed half billion people in developing countries

- ❖ Protein deprivation damages human health among the poor in the Third World
  - ...where 'hunger' often translates to shortage of protein
  - For example, it hurts brain development in children
- ❖ Cotton is very toxic due to gossypol, a terpenoid aldehyde
  - 2,2′-bis-(Formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene)
  - ...which can only be digested by ruminant microflora, but only to a certain level
- ❖ Cottonseed is rich in protein (22 %) of very high quality
  - ... gone to waste hitherto, due to its high gossypol content
  - ...though cottonseed (44 billion kg/year) could provide new, high-protein food for 500 million people annually
- ❖ Edible cottonseed has now been bred using RNAi
  - ...a gene silecing method awarded with Nobel prize in medicine in 2006
  - ...though it has been used in plant GM since decades ago
- ❖ Production of gossypol was only silenced in the seed
  - ...so that the indigenous chemical defence against pests was successfully retained in other plant parts
- ❖ That is not possible applying "traditional" breeding methods
  - Gossypol production was silenced by traditional mutagenesis in experimental cotton lines already in 1970's
  - …with the consequence that such defenceless plants were destroyed altogether by pests and diseases in the field

- Sunilkumar et al (2006). Engineering cottonseed for use in human nutrition by tissue-specific reduction of toxic gossypol. PNAS 103: 18054–18059
- Field trials: http://agnews.tamu.edu/showstory.php?id=1399

Domesticating extra wild species for cultivation...

J. Tammisola©

- Arctic bramble (*Rubus arcticus*)
  - the most aromatic berry in Europe (Línné 1762)
- Rare species with declining populations
- First cultivation trials by Linné
- Breeding attempts since 1920's
  - with scanty results

☞ Tammisola (1988) *J.Agric.Sci.Finl.* 60: 327–446

...or achieving poor compromises by classic crosses?

- Arctic bramble is unreliable and tedious to cultivate, due to its ”primitive” features
  - it only thrives in the Far North (latitudes > 60º), and
  - cannot tolerate weeds (is a weak competitor)
  - is susceptible to fungal and virus diseases
  - is self-sterile (many varieties need to be grown mixed)
  - its soft berries cannot be picked mechanically
- More robust growth, ”tolerance to South”, and disease resistance could be gathered from the American-Asian sister subspecies (ssp. *stellatus*)

Distortion of the aroma by combining unknown genes at random

- Crosses and backcrosses between these two ”sister” brambles were made during decades in Sweden
  - resulting finally in a more southern, more robust and less disease-prone bramble type (”noble bramble”)
- Alas, the unique arctic bramble aroma was lost!
  - consequently, ”noble bramble” berries (albeit their still premium aroma) are not accepted to genuine ”Mesimarja” liqueur by the industry
- The lesson: saviour traits should, for caution, be introduced in a purified form (using gene technology)

☞ Pirinen et al. (1998) *Agric. Food Sci. Finl.* 7: 455–468

”Goorrant” (”karukka”) is a hybrid between gooseberry and blackcurrant. 1.

Karukka, Helsinki 2009.

”Goorrant” (”karukka”) is a hybrid between gooseberry and blackcurrant. 2.

Karukka, Helsinki 2006. © J.Tammisola

- Traditionally in plant breeding, all the thousands of genes from two different plant species are at liberty to be combined together without official control
- Whereas transferring just a single, carefully studied gene out of this mess in a purified form...
  - applying precise modern methods
- is strictly constricted with legal regulations
- ...launching tough and extremely costly demands (Tammisola 2006)

The livelihoods of half billion people depend on banana, the most important fruit in the world

Juvenile banana fruits, trailing the male inflorescence. Rhodes, Greece, 2009. © J.Tammisola

- In Uganda, Burundi and Rwanda people eat 250–400 kg of bananas a year (BI 2010)

Is the evolution of devastating new races of fungal diseases going to eradicate our current commercial banana varieties?

Black Sigatoka ruins banana leaves

- ...just as that destroyed the far better and sweeter ”smiling banana” (’Gros Michel’) half a century ago (Pearce 2003, Bennett & Arneson 2003, Ploetz 2005)

…though, in rich countries, these novel pathogens can still in part be controlled with frequent fungicide sprayings

- 20–40 (up to 70) sprayings per year may be required in banana plantations
  - That is too costly for small producers in the Third World
  - ...since fungicides are expensive and should preferably be spread by plane
  - Sprayings also burden the environment
- Furthermore, the efficiency of the fungicides tends to impair with time
  - ...due to resistance evolving in the pest
- Accordingly, developing disease resistant banana varieties would be fundamental to the livelihood
- Anyway, owing to the sterility of edible banana varieties, hardly any progress could be achieved with four decades of conventional breeding (Pearce 2003)

Could grocery store bananas ('Cavendish') be rescued with wild bananas?

Wild bananas are packed full with hard seed

- Commercial banana varieties are seedless, parthenocarpic triploids
- ...whilst wild banana species carry inedible fruits (virtually devoid of flesh but full of seed)
  - ...called "tae manu" (animals feces)
- Anyhow, wild bananas hold resistance genes
- ...which could be utilized in commercial banana varieties
  - ...though, in practice only with genetic modification

Forty years of wasted efforts in disease resistance development using retarded breeding methods

- 10 hectares of ’Cavendish’ bananas were force-crossed by hand pollinations with resistant Asian wild bananas
  - 400 000 kg of banana fruits were mashed through sieves
  - In total 15 seeds were found
  - ...of which 4 ones could be germinated
- These few species hybrids were then backcrossed with wild bananas
- ...finally yielding one seedless hybrid banana resistant to two severe diseases (Black Sigatoka & Fusarium wilt)
- Not quite unexpectedly:

  (given the thousandfold unnecessarily dirty,

  ”conventional” method, i.e. crosses)

  - That flagship of traditional plant breeding
  - ...is sour, and
  - ...primarily tastes of apple (!)

Old top varieties: to be lost with crosses, or fixed with genetic modification?

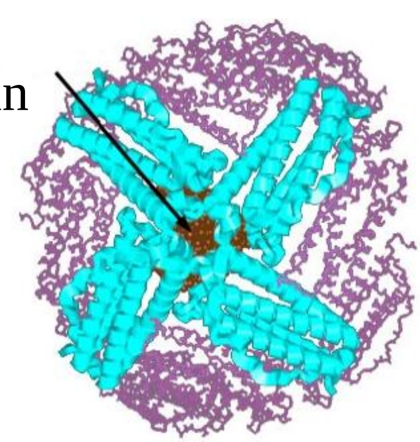
- The fiasco of ”Sour Banana” came of inserting thousands of unknown, unnecessary and disadvantageous genes in banana genome
  - ...though only certain prescribed genes (for disease resistance) would have been needed
- Thus, no responsible plant biologist would apply such dirty and unpredictable, traditional ”black box” methods of breeding for the problem any more today (Tammisola 2006)
- Instead of a cacophony of genes, only the chosen few useful ones should be transferred in crops
  - ...purified from any hitchhiker genes whatsoever
- ...using latest precise genome modification methods such as gene targeting (Shukla et al 2009, Townsend et al 2009, Porteus 2009)
- Popular old varieties, such as ’Gros Michel’, could be resurrected on grocery store shelves by fixing their faults (whilst retaining their top qualities intact) with GM (see GM Cavendish in Australia, Promusa 2011)

’Gros Michel’ banana modified with two rice genes for resistance against Black Sigatoka: Field tests are going on in Uganda

- Chitinases are involved in plant defence: they have anti-fungal properties
- ’Gros Michel’ banana variety was modified with chitinase genes Rcc2 and Rcg3 from rice
- Laboratory evaluation of GM banana showed very high resistance to Black Sigatoka (Kiggundu et al 2008)
- GM lines are being tested in the field for the stability of chitinase expression in different environments (Dauvers 2007)
- Meanwhile, progress in genomics by initiatives such as the *Global Musa Genomics Consortium*
- ...raises the possibility of using genes found in wild banana species instead of genes from more distantly related plants
- Such ‘cis-genic’ crops may be more acceptable to laymen scared of crossing species borders
  - though such borders are commonly crossed in the Plant Kingdom (Tammisola 2006)

Biofortification of banana for increased levels of pro-vitamin A and iron 1.

- The major micronutrient deficiencies in Uganda are:
  - Iron deficiency anaemia (IDA)
  - Vitamin A deficiency (VAD)
- In 2006:
  - 15-32% of children < 5 years had VAD
  - 13-31% of women had VAD
  - 50-80% of children < 5 years had IDA
  - 32-64% of women had IDA
- Biofortification targets in banana fruit:
  - **four-fold** increase in **pro-vitamin A**
  - **three-fold** increase in **iron** content
- Increased iron accumulation in fruit
  - increased expression of ferritin +/- iron transporters (FRO 2 and Irt 1)
  - Iron stored as mineral inside ferritin



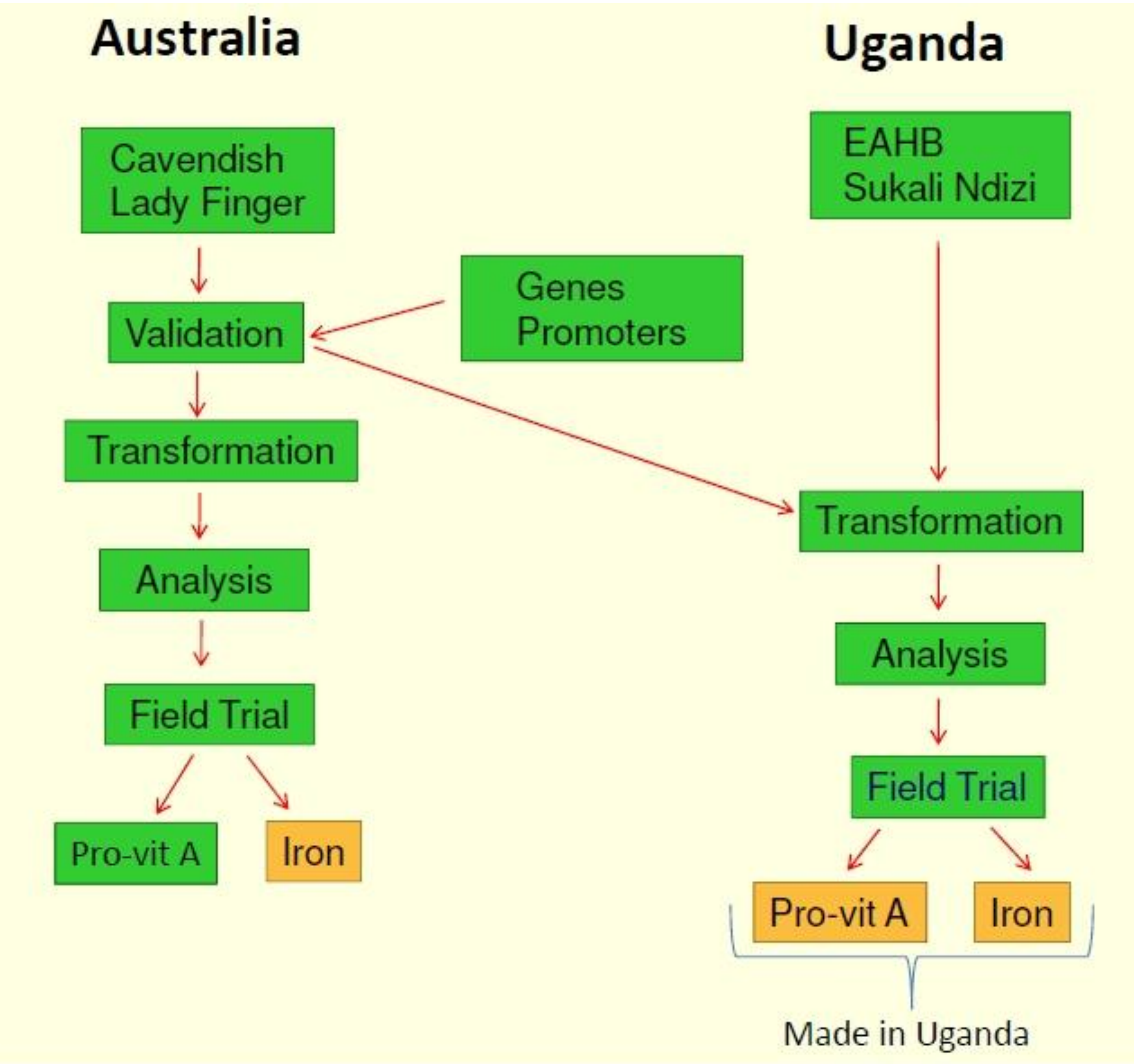
(Rob Harding, Brisbane Univ. of Technology, Australia)

Biofortification of banana for increased levels of pro-vitamin A and iron 2.

- Two core parallel projects: Australia (QUT) and Uganda (NARO)
- Transformation and characterisation of
  - EAHB (cooking banana) and
  - Sukali Ndizi (dessert banana) at NARO
- Test in field trials
- Increased pro-vitamin A (pVA) biosynthesis in fruit
  - the Golden Rice strategy
  - increased expression of one or more enzymes in the pVA synthesis pathway - phytoene synthase (psy) +/- carotene desaturase (crtI)

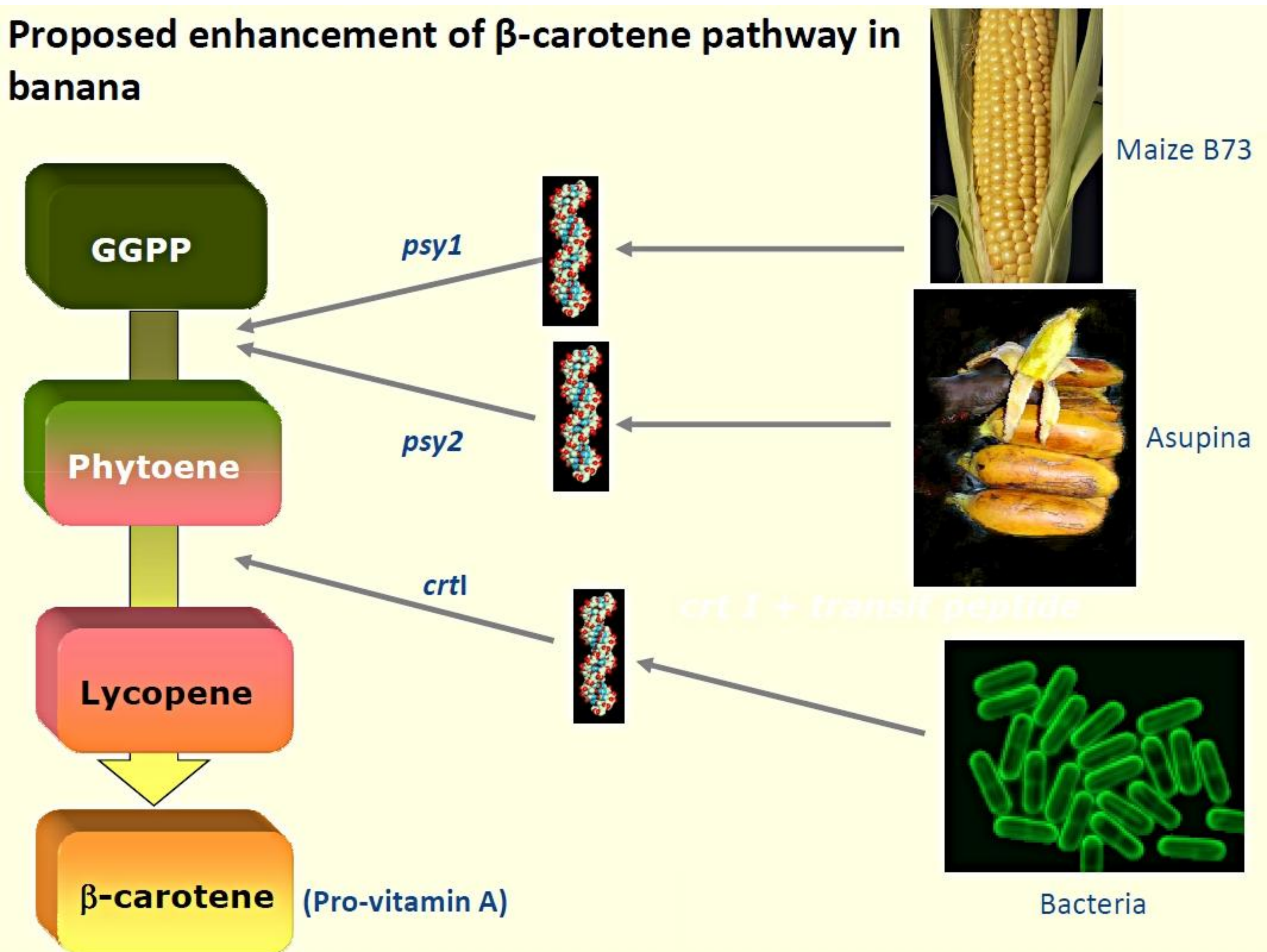
(Rob Harding, Brisbane Univ. of Technology, Australia)

Biofortification of banana for increased levels of pro-vitamin A and iron 3.



(Rob Harding, Brisbane Univ. of Technology, Australia)

Biofortification of banana for increased levels of pro-vitamin A and iron 4.

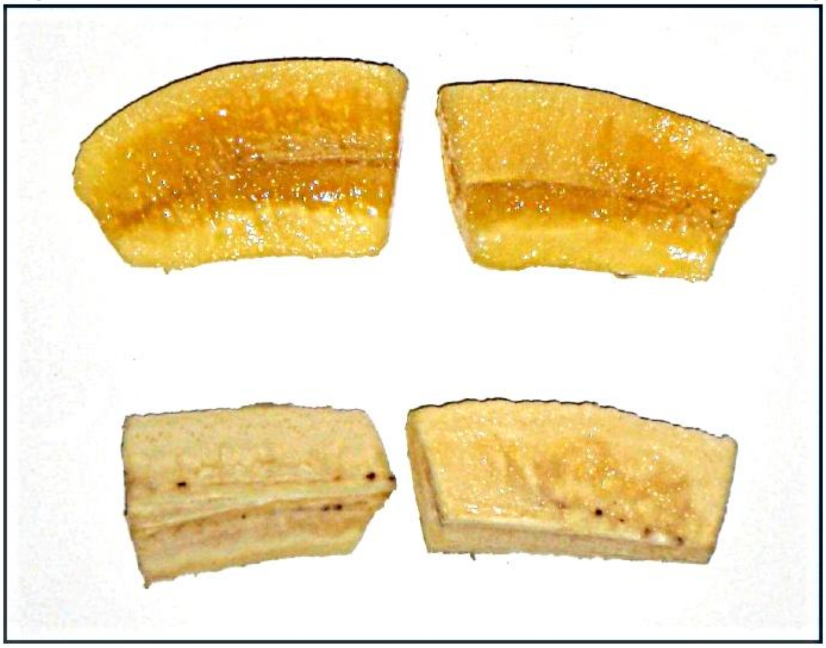


(Rob Harding, Brisbane Univ. of Technology, Australia)

Biofortification of banana for pro-vitamin A, selections from first field planting

Full Ripe Fruit

(First GM fruits from field trials, Feb. 2010)



Carotenoid content from full green banana fruit			
	Carotenoids ug/gdW	Increase over control (x)	Promoter Transgene
FT-155	3.92	NA	Control Cell line
FT-201	15.63	3.99	E1P1
FT-207	18.92	4.83	E1P1+UC
FT-246	23.83	6.08	E1P2
FT-296	25.28	**6.45**	UP2
FT-330	20.54	5.24	UP2
FT-342	22.95	5.85	E1P2

(Rob Harding, Brisbane Univ. of Technology, Australia)

Wheat that repels aphids by producing aphid alarm pheromone (EBF)

- The volatile sesquiterpene (*E*)-β-farnesene (EBF) is used by aphids under attack as an **alarm pheromone** or **signal**
  - causes other aphids to stop feeding and move away from the source
  - acts as a repellent to colonising aphid morphs
  - emission of EBF would also be expected to cause increased foraging by predators and aphid parasitoids (Beale et al. 2006)
- EBF is produced by some plants, e.g. peppermint, as a defence against aphids
- Wheat lines producing EBF are developed by Rothamsted Research, UK
  - Initiating non-toxic, eco-friendly pest control?
- High-level protection against aphids has been recorded in laboratory trials
  - without any insect-killing chemical
- Field trials started in 2012 (Defra 2013)
  - hit by anti-science vandals, as usual...
- For details, see the application document: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/206733/11-r8-01-part-a1.pdf

Insulin from plants resolves world insulin crisis?

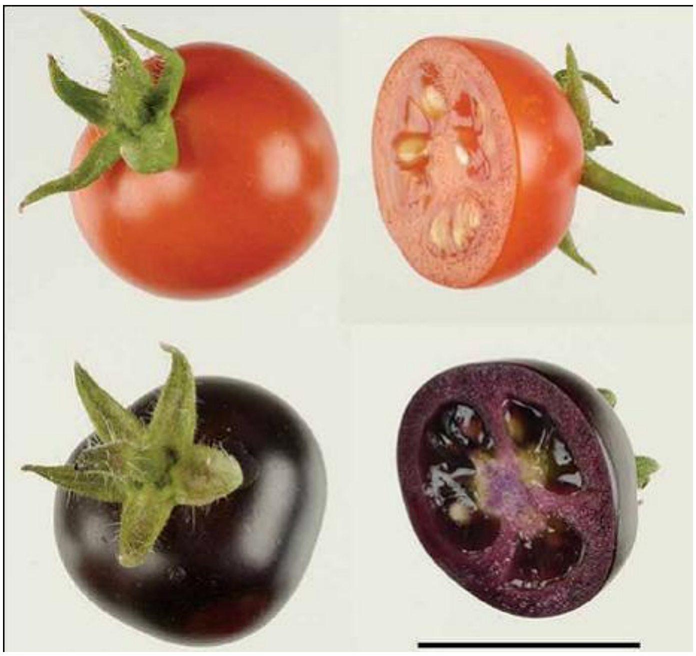
- Human insulin precursor is produced in high amounts in GM safflower (*Carthamus tinctorius*) seed oil
  - ...from which it can be readily extracted in a pure form and processed into active insulin
- High yield & dramatically lower production costs → affordable insulin for all diabetics in the Third World
  - Three commercial farms (in total 6 500 ha) might suffice for the entire world
- Phase 2 clinical trials (safety) have been passed, and Phase 3 trials (efficiency) are going on in Canada (2010–)
- Drug approval (FDA) is awaited shortly thereafter http://www.sembiosys.com/Products/Diabetes.aspx

GM insulin saved diabetics and whales

- Insulin was at first obtained from swine
- ...but from whale spleen after WW II
  - Special whaling fleets were founded by pharmaceutical companies, e.g. Nordisk
- Animal insulin carries the risks of zoonoses and insulin allergy
- Human insulin from GM yeast was developed by Hoechst in Germany
- Hoechst insulin factory should have started production in 1984
- ...but anti-science movements in Germany succeeded in retarding its permission for 14 years
  - ...by all kinds of administrative trickery
- ...rendering it ”The most expensive biotechnology museum in the world”
- Meanwhile world insulin markets were safely lost to American and Danish drug companies

☞ http://www.nature.com/nbt/wilma/v16n5.894052109.html

Anthocyanin tomatoes prevent cancer



- Two genes from snapdragon were bred in tomato
  - transcription factors (regulate functioning of other genes)

- Anthocyanins were accumulated in tomato fruits to as high concentrations as in blueberries
- Blue tomatoes helped cancer mice live one quarter longer than red ones did (Butelli et al 2008)
- ...though also red tomatoes are known to prevent cancer
  - owing to their red pigment (lycopene)

Breeding long-chain ω3 oils into crop plants – health for the needy

- Long-chain ω3 fatty acids (EPA, DHA) prevent heart diseases
  - ...and may even reduce death rates as much as statins do (Stanley 2006, *Lipid Tech.* 18: 158)
- Fish products alone have so far been the dietary source of these healthy fatty acids
- ...but supply of fish is declining in the world
  - due to exhaustive fishing and consequent restraints
- Hence, plant breeders are developing oil crops that could provide recomended daily allowance (RDA) of long-chain ω3 fatty acids for all people, especially
  - the poor ones, often relying on plant-based diets
  - vegans
  - people allergic to fish

Breeding long-chain ω3 oils into crop plants – health for the needy

- Fish cannot produce EPA or DHA themselves but receive those fatty acids from sea algae
- Mosses and algae can make long-chain ω3 fatty acids but flowering plants cannot
  - crop plants can only synthesize short-chain types of ω3 fatty acids
  - ...with merely scanty benefits to human health, because only a negligible fraction of those acids is converted to the long-chain ones in our cells
- The key gene was purified from a seaweed and inserted in soybean and oilseed rape
- Field trials proved that high amounts of EPA and DHA are generated in the crops
  - 1 ha of GM soybean yields these oils as much as 30 000 salmon
- Clinical trials proved the efficiency of heart-nurturing long-chain ω3 soy oil in human use (American Heart Association 2009)
- Awaited dates of release in grocery stores: 2012 in USA (and 2015 in Australia, CSIRO 2009)
  - **Solae Soymega™SDA Soybean Oil:**
  - **http://www.solae.com/Soy-Solutions/Innovation-Research/Omega-3-Soymega-Solution.aspx**

Relieving allergies with plant breeding 1.

- One fifth of people living in industrialized countries suffer from pollen allergy
- Birch pollen is the main allergen in the Nordic countries
- ...whereas Japanese cedar (*Cryptomeria japonica*) commonly causes much more severe symptoms in Japan

Japanese cedar in Azores. Dec. 3, 2006. ©Gaspar Avila

Crops preventing or curing allergy or asthma... 2.

- In industrial countries, one in five persons suffer from pollen allergy
- Birch pollen is arduous in the North, whereas Japanese cedar causes strong symptoms in Japan
- A peptide combining the immunologically most essential patches of two major allergens in cedar pollen was synthesized (by gene technology)
- A synthetic gene coding for the peptide was then bred to rice, and the peptide was produced in rice seeds
- Cedar allergy was prevented in mice, when they were fed in advance with such seeds
- ...signifying edible vaccine against pollen allergy, without the danger of anaphylactic reactions

☞ Takagi et al. (2005). *PNAS* 102: 17525–17530

- The safety of the cedar allergy vaccine in development has recently been proved with Macaques (Domon et al 2009)

...without injection needles 3.

- Similarly, onset of experimental asthma (caused by sunflower seed albumin) could be prevented by oral vaccination
  - mice were fed in advance with lupin seeds, which were bred to contain sunflower seed albumin
  - such edible vaccine prevented the onset of sunflower asthma in the mice...
  - ...even after heavy dusting with the asthma launching protein at issue

☞ Smart et al. (2003). *J. Immunol.* 171: 2116–2126

Soybean allergy is common 4.

- Soybean is one of the ” big eight” food allergen sources
- Soy occurs in many processed foods, and it is hard to avoid
- A severe but rare reaction is anaphylactic shock
- Soybean seeds contain 1 400 different proteins
- About 7 of these seed proteins commonly cause allergic reactions in adult human population in USA
  – still extra ones may rise antibodies in babies (but do not cause symptoms in adulthood)

Breeding less allergenic soybeans... 5.

- Scientists try to remove the core of allergenic proteins from soybeans
  - onset of new allergy cases agains such proteins could be decreased or prevented
  - regarding inadvertent soy exposures, the allergic reactions of sensitized persons would be less severe
- The immunologically most dominant allergy protein (P34) in soy was removed by silencing its gene with gene technology
  - P34 protein causes more than 65 percent of soybean allergy reactions in USA
  - silencing the undesired gene did not harm the plant’s agronomic characteristics

☞ Herman et al. (2003). *Plant Physiol.* 132: 36–43

☞ Herman (2003). *J Exp Bot* 54: 1317–1319

...gene by gene 6.

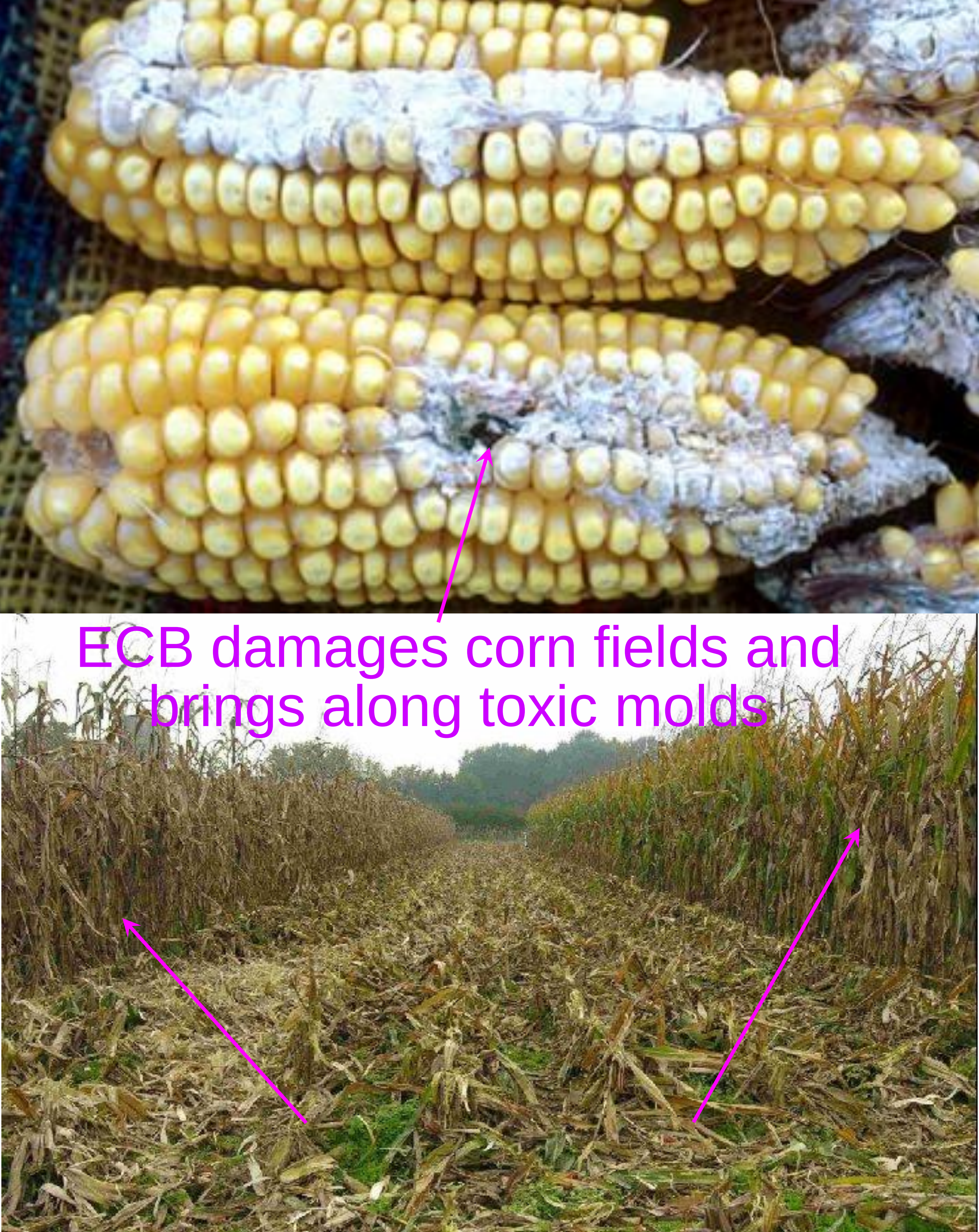
- Second most important allergenic protein was found lacking in one soybean line in gene bank materials
- The desired characteristic can be combined with the foregoing hypoallergenic trait
  - by classic crosses, because
  - soybean varieties are self-pollinated pure lines
- Work is underway for silencing the third most important allergen in soy (by gene technology)

GM vaccine against birch pollen allergy is in development 7.

- In the Nordic countries, 98 % of birch allergics only react to one birch pollen protein (Bet v 1)
  - In Central Europe, people may often react to Bet v 2 as well
- Thus, regarding vaccine development, the situation in birch is somewhat simpler than in Japanese cedar
  - ...from which at least four allergenic proteins have been reported so far
- No edible vaccine against birch pollen allergy is available yet
  - ...but efficient and safe injectable vaccine for Bet v 1 –caused birch allergy is already being developed
  - ... based on genetically modified vaccine protein (fusion peptide) (Mahler et al 2004)

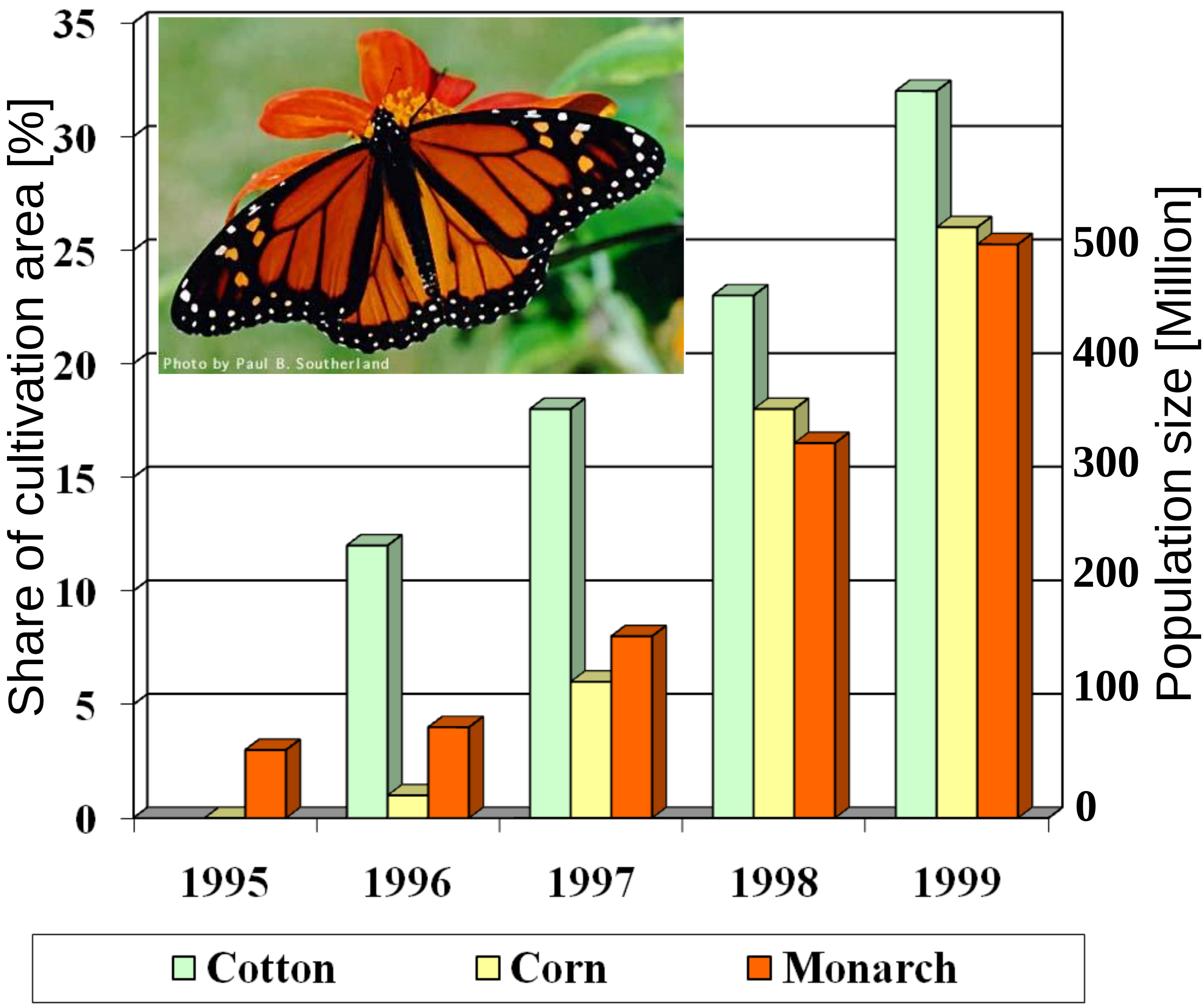
European Corn Borer increased mold toxins 100-fold in Italian maize cobs

- Cobs damaged by ECB larvae are often conquered by poisonous molds (*Fusarium*)
- Their toxins (e.g. fumonisin) are deleterious to human health:
  - they cause cancer and damage liver, kidneys, nervous system and developing embryos (Marasas et al 2004, Gelineau-vanWaes et al 2005, 2009)
- Developmental disorders such as *hydrocephalus* and *spina bifida are* much more common among neonates in ”Tortilla-zone” countries
  - ...where their mothers are often exposed to poor-quality corn products during pregnancy
- Fumonisin toxin content is radically reduced in the cobs of ECB-resistant Bt-maize varieties
  - especially so in South Europe



Results of field release in Italy, left conventional, right transgenic insect-resistant maize
http://www.agbioworld.org Data produced by the University of Milan, Italy

Insect-resistant Bt crops supported the growth of Monarch populations in USA in1996–99



- USDA (1995–2000)
- Carpenter JE, Gianessi LP (2001). Agricultural Biotechnology: Updated Benefit Estimates. Nat. Center for Food and Agric. Policy, Washington www.ncfap.org
- *Monarch Watch* vol. 3–8 (1995–2000), Univ. of Kansas www.MonarchWatch.org

Producing Autumn Blight ...or potatoes?

– Blight resistance is being introduced from wild potatoes with genetic modification (Song et al 2003)

Potato late blight – worst potato disease globally

- Killed million people for hunger in Ireland during 1845–50
- Received compatible mating partner from America a couple of decades ago
  - and started sexual reproduction...
  - so that its genetic diversity and evolution are enhanced, and
  - blight epidemics grow worse in EU
- No true resistance is available in cultivated potato (*S. tuberosum*)
  - but only diverse grades of vulnerability
- Race-specific resistance cannot sustain...
  - but collapses every time when new blight races are generated by evolution

Broad-spectrum resistance to potato late blight is on offer from a wild species

- A wild potato (*S. bulbocastanum*) is resistant to potato autumn blight
- Gene for resistance was searched for, purified, and bred in cultivated potatoes by gene technology
- Potato lines being tested showed resistance to all known blight races
  - including a ”super race” that can overcome all race-specific resistances
- The trait could not be retrieved by old means, because of
  - cross barriers (unequal ploidy levels)
  - exhaustingly long time to be needed
  - risk of toxins being generated in cultivated potato due to unwanted (hitchhiking) genes from wild potato

Popular old varieties can be rescued and improved

- Russet Burbank is a favourite American potato variety since 100 years
- It is still grown on almost half of the total potato area in USA
- Popular clonally (vegetatively) propagated plant varieties can be kept competitive for the future
  - by revising their obsolete (bottleneck) characteristics or enriching their vital traits with the help of gene technology
- Burbank potato can be turned blight resistant ('BR Burbank')
  - ...and the European favourites alike

Blight resistance benefits environment and food quality

- Productivity and tuber quality are greatly deteriorated by blight infection
- In temperate climates, controlling blight often requires 10 fungicide sprayings

– ...but in hot areas, suh as Mexico or Asia, up to 25 sprayings may be needed per season

– ...which badly hinders moving from rice to water-saving potato cultivation in the tropics

- BR potatoes would save EU each year from
  - 860 million kg of potatoes being wasted
  - 7.5 million kg of fungicides* to be used
    (*measured in active ingredient)

☞ Phipps & Park (2002). *J Animal Feed Sci.*11: 1–18

☞ Gianessi et al (2003). Potential impact for improving pest management in European agriculture. Potato case study. NCFAP

Genetics of aromatic cereals was resolved

- Thai scientists found out the genetic basis of fragrance in cereals in 2005 (Bradbury et al 2005, 2008, Kovach et al 2009)
- In ordinary cereals, the gene for non-fragrance is functioning
  - whereas in aromatic rices (basmati and jasmine) it is silenced by a mutation
- The gene was purified
  - and patented: for keeping its intellectual property rights in the developing country
- Wheat has altogether 6 non-fragrance genes, and consequently all of these cannot be silenced by old means:
  - Breaking one specific gene traditionally with chemicals or radiation may succeed, by investing plenty of time and efforts
  - ...but breaking all 6 ones by blind chance is simply not possible

Breeding aromatic wheat

- Though, aromatic wheat can be bred at a couple of steps with genetic modification
  - all 6 non-fragrance genes can be silenced in a focused way simultaneously
    - » with RNA interference (Vince 2006)
  - or sequentially in groups, at a few steps
    - » with targeted mutagenesis (Shukla et al 2009)
- The real challenge may be obtaining EU clearance for importing fragrant wheat
- Such permission is obligatory in practice
  - even if the product is not aimed at EU markets
  - namely, without such prior permission, import bans are launched even if minuscule amounts of the fragrant crop is mixed with the wheat imported in EU (Tammisola 2006, chapter 10)

Precautionary Principle is misused in EU

- ”Where there are threats of serious or irreversible damages,
  lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures
  to prevent environmental degradation”
  (1992 Rio Declaration on Environment and Development)
- The Principle aims at facilitating the *early use* of necessary new means under development for environmental protection
- Though in EU, it is inconceivably being applied to *preventing* the mobilization of more efficient and safe methods based on modern sciences
- ...which would be invaluable for finding solutions to the ”infernal” problems we shall meet with conserving key natural resources, taking account of the pace of major changes going on in the world

Many breeding traits are ecologically benign

- Quality traits improving the usability of plant products for human needs
  - are generally not prone to help the plant invade or survive better in natural ecosystems...
  - ...the more than similar traits bred before by old means
  - but are as a rule discarded from ecosystems due to natural selection
- Whereas adaptive breeding traits deserve more careful scrutiny for environmental effects (Tammisola 2009)
  - because such traits might become more common (though not necessarily dominating) in natural plant populations

["The Classic"]
1989 Statement of Eucarpia* on Risk Assessment Regarding the Release of Transgenic Plants

1. It is the prime competence and responsibility of every research worker to evaluate potential risks of his research and to find ways to control these.

2. The plant is a relatively easy organism to control. Many crop plants are fully dependent on man for their existence.

3. In assessing risks the potential gene flow is crucial. This is determined in amount by the mating type and by the degree of taxonomic relationship. Much knowledge on these phenomena is already available in the literature.

4. Secondly, the effect of the gene is relevant and not the way it was introduced into the genome.

5. It should be kept in mind that well-defined genes, such as those transferred to plants by molecular techniques, can precisely be identified and controlled at the molecular level. However, their phenotypic expression must always be monitored most carefully.

6. There are genes which a priori are known to be harmful. These are not to be transferred into crop plants.

7. Presently, case studies with the release of transgenic plants are underway in several countries. All results should be fully published.

8. Eucarpia has established a working group of competent scientists on the subject of risk assessment for the release of transgenic plants.

*European Association for Plant Breeding Research