New and emerging applications in biotechnology

Challenges, potential and economic impact on European agriculture

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Crop plants 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

Crop plants have been modified

- 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
- Toxic and harmful chemicals have decreased in food crops
- Nutritional contents (e.g. essential amino and fatty acids) have been adjusted towards human requirements

Art of breeding

- Many traits still remain to be improved
- Art is artificial beauty
 - and plant breeding was traditionally hailed as classic art by us breeders
- Recent breakthroughs in molecular biology and genetic understanding have
 - evolved plant genetics, physiology and breeding to true sciences
 - much improved the precision, efficacy, predictability, safety and application potentials of plant breeding
- Consequently, hundreds of biological inventions lay on university shelves
- ...waiting for financial and social support
 - to be finalized and taken advantage of
 - to push through the obstacles of product legislation

Novel prospects and challenges

- Quality, Nutrition, Health, Environment, and Bio-economy
 - five core drivers in the rapidly changing world

8 characteristic cases:

- 1. Aromatic wheat
- 2. Omega-3 plant oils
- 3. Inulin beet
- 4. Crops preventing or treating allergy or asthma
- 5. Blight resistant potato
- 6. Rootworm resistant corn
- 7. Enhanced starch potatoes
- 8. Better trees for bio-economy

Coping with rapidly changing environments

- Climatic change alone would ask plant scientists a herculean task:
 - update the existing plant varieties to be suited to changed conditions
 - ✓ breed a new generation of staple crops provided with crucial new traits, such as
 - drought, heat, salt or flooding tolerance
 - much more solid resistance to possibly ever more prolific plant pests and diseases
- Such amendments must likely be attained only to retain the current yield levels and efficacy in food production
 - improving food security would presume somewhat more creativity, and slightly more positive attitudes towards biosciences

Harnessing bio-economy asks for new achievements in biological efficiency

- Achieving high productivity is a key question for reaching any true sustainability in bio-economy (biofuels included)
- Biotechnological solutions successfully competing with old technologies in yields, energy use and costs
 - have to be created within a few years/decades
 - pose a great challenge (even) for EU
- Case: clubroot resistance in oilseed rape (could be bred by gene technology) would allow great increases in rapeseed oil production
 - resistant oilseed rape could be cultivated in consequtive years (whereas the crop is grown only once in 5 years today)

1. Genetics of aromatic rices was resolved

- Thai scientists found out the genetic basis of fragrance in cereals
- In ordinary cereals, the gene for <u>non</u>fragrance is functioning
 - whereas in aromatic rices (basmati and jasmine) it is silenced by a mutation
- The gene was purified and patented, to keep its intellectual property rights in the developing country
- Wheat has altogether 6 non-fragrance genes, and so all of these cannot be silenced by old means...
 - breaking one specific gene traditionally with chemicals or radiation may succeed, by investing much time and efforts,
 - but breaking all 6 ones by chance is not possible

Breeding aromatic wheat

- ...whereas aromatic wheat can be generated at one step by gene technology
 - all 6 non-fragrance genes can be silenced simultaneously in a focused way (e.g. with antisense technique)
- The real challenge may be obtaining EU clearance for receiving fragrant wheat
- Such permission is obligatory in practice
 - even if the product is not aimed to EU markets
 - namely, without such prior permission, import bans are launched even if minuscule amounts of the fragrant crop is mixed with the imported batches
- The Bradbury et al (2005). Plant Biotech. J. 3:363-370

2. Omega-3 plant oils – health for people at risk

- Long-chained omega-3 fatty acids (EPA and DHA) are known to prevent cardiovascular diseases such as heart attacks
 - they may in fact decrease mortality as much as statins (Stanley 2006, *Lipid Tech.* 18: 158)
- These fatty acids are only obtained from fish foods currently
- However, seas are already overfished, and their fish supply does not suffice for all mankind
- Therefore, plant breeders are trying to develop crop plants which could provide us with the recommended daily intake of long-chained omega-3 oils
- Such plants could especially help world's poor (often relying on plant-based diets), fish allergics and vegans

Breeding oil crops for long-chained omega-3 oils

- In fact, fishes acquire their long-chained omega-3 oils from algae
- In plant kingdom, mosses and algae can produce long-chained omega-3 oils, whereas flowering plants cannot
 - crop plants can only prepare short-chained ones with scarce benefits
- The required gene was purified from an algal species, and bred to conventional oil crops by gene technology
- Field tests are going on, and good levels of EPA and DHA are obtained in the experimental crop lines
- First heart-protecting omega-3 plant oils are awaited to shops in 2011 in America

Taming extra wild species for cultivation...



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- 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 > 60°),
 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

3. Inulin beet

- Inulins are a group of health-promoting fructan polysaccharides
- These are formed in a few plant species,
 e.g. chicory (*Cichorium intybus*)



Chicory in Sunny Beach, Bulgaria. J.Tammisola©

Inulin as a funtional preparation

- Short-chained inulins can be utilized as non-caloric sweeteners
- Long-chained ones serve as food fibers stabilizing digestion
 - health benefits are shown both in human and animal studies
 - pigs generate less odours and methane (a potent greenhouse gas), and thereby
 - pollute less, and
 - produce better-tasting meat
- Inulins for medicinal uses are currently produced by bacterial fermentation or from chicory
 - but the product is too expensive for routine use, especially in feed

Breeding inulin crops

- Sugar is a bulk commodity produced considerably in excess in the world
 - e.g. half of the Finnish sugar processing facilities were run down recently
- Extra capacity could best be switched to higher-valued special products
- Regarding large scale uses, chicory is an uneconomic crop for production...
- ...but high-yielding sugar beet could be bred to produce inulins
- Two genes necessary in their synthesis were bred from Jerusalem artichoke to sugar beet
 - and the bulk of the customary sucrose was replaced by fructans in the beets

 Sévenier et al. (1998). Nat.Biotech. 16:843-846; (2002). J.Am.Coll.Nutr. 21:199S-204S

4. Crops preventing or treating allergy or asthma...

- 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

...without injection needles

- 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

- 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...

- 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

...gene by gene

- 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)

Resistance to plant diseases

- Disease resistant plants could offer a great reduction of environmental stresses in (or due to) agriculture
- Plant diseases often cause, in vain,
 - big losses of yield
 - wastage of field area, chemicals, energy and work
 - poor product quality, and
 - generation of noxious natural chemicals (defence metabolites, carcinogenic toxins) in the crop

Breeding for virus resistance

- Notably, resistance to virus diseases is quite often lacking in the breeding populations of food crops
- Resistance could be bred, however, via kind of genetic vaccination*, by gene technology
- Most universal advantages could be achieved in tropical areas and developing countries
- Losses due to a virus disease may be massive (often the bulk of the yield)
- Resistant crops allow for reductions in insecticide sprayings (hitherto necessary to control virus vectors)
- Cases: virus resistant squash, papaya, cassava etc.
- * RNA interference (see Nobel Prize 2006) applied to prevent virus functioning in plants

Breeding for fungal resistance

- Fungal diseases must generally be controlled by (often expensive) fungicide treatments
- Molds in plants may produce highly carcinogenic toxins (e.g. aflatoxin)
- Hidden mold exposures are hard to avoid when consuming agri- and horticultural products
- E.g. mold resistant strawberries have been bred in academies but are not publically available, because...
- ...challenges for commercialization are prohibitively hard, due to heavy regulatory load (*ergo* costs), regarding
 - public sector
 - minor or local ("orphan") crops
 - clonal varieties (such as strawberries) with only limited area of cultivation per variety

Hidden infections in agricultural products may expose people to toxins



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 mold has infected apple heart through apple worm hole

Breeding for bacterial resistance

- Bacterial diseases cannot often be controlled but by using antibiotics
 - with their recorded risks of evolving antibiotic resistance in harmful bacteria
- Bacterial fire blight in apple gardens can only be controlled by streptomycin treatments today
 - constituting the bulk of antibiotics use for crop production in the West
- Fire-blight resistant derivative of Royal Gala apple has been bred
 - final part of the story depends on politics...



Tiu et al. (2001) Agra Hortic. Proc. 560: 95–99

5. Potato varieties can be turned resistant to late blight



Song et al. (2003). *PNAS* 100: 9128–9133

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,

– 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
 - in hot areas, suh as Mexico, up to 25 sprayings per season may be needed
- Blight resistant potatoes would save EU each year from
 - 860 million kg of potatoes being wasted
 - 7,5 million kg of fungicides to be sprayed (expressed as 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

Precautionary Principle

- "Where there are threats of serious or irreversible damages, <u>lack of full</u> <u>scientific certainty shall not be used</u> <u>as a reason for postponing cost-</u> effective measures to prevent environmental degradation"
- 1992 Rio Declaration on Environment and Development

6. Western Corn Rootworm came and conquers Europe

Veni, vidi – vici?



Photo: Whitney Cranshaw

Diabrotica virginifera is a nice beetle – and a devastating alien species

...causing severe yield losses



- Rootworm larvae cut corn roots, and stems fall down
- Spread of adult beetles cannot be halted with sprayings



- Irrigating soil with strong insecticides is only half efficient against the larvae
 - non-selective means also damage non-target soil organisms

Rootworm resistant corn is in cultivation elsewhere

- Resistance is based on expressing beetlespecific Bt protein in corn roots
- Comparatively high specificity (narrow target spectrum) allows focused control of nibbling rootworm larvae
- Resistant plants provide pointwise control which is generally to be preferred over less focused techniques such as sprayings
- Import of rootworm resistant corn for food and feed uses is allowed in EU
- Its cultivation is projected to cause a major drop in insecticide use on corn fields in USA

Western Corn Rootworm continues spreading...



(80 km/year)

...and calls for biologically valid emergency measures

- Rootworm is an invasive and devastating alien pest species
- Pursuant to precautionary principle, even lack of full scientific certainty could not justify postponing proper preventive measures
- Giving up is not the solution even in the areas of established epidemics
- Rootworm resistant corn could attenuate the pest population from epidemic to manageable sizes, and thereby

help all growers, organic ones included

- reduce the pest's spreading pressure
- Long-term reductions in pest population densities have been gained by growing Bt corn and Bt cotton in USA

Carpenter & Gianessi (2001), Carrière (2003)

7. Enhanced starch potatoes and ecological starch paper

- Ordinary potatoes contain two types of starch: amylose and amylopectin
- The latter suits many technical applications (e.g. glues and paper) much better
- Amylopectin potato was bred by silencing one native potato gene by gene technology
- Application to permit its growth and use has queued in EU since 1996
 - and may be cutting the tape before next spring sowings

Breeding more efficient starch potatoes...

- Full yield of quality starch is accompanied by environmental benefits
 - energy and chemicals are being saved, because better starch needs less modification by chemical means
- Boreal Plant Breeding is developing industrial potato for substantially higher starch content by gene technology
- Field tests are going on for the third year in Finland
 - only sabotaged in autumn 2005
- Regarding bio-economy, the most sought-after final outcome might be found by combining high starch content with desirable quality
 - e.g. by traditional crosses between these two industry potatoes (or by gene technology)

Boreal starch potato in field tests (2004)



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...may enable transition to environmentally friendly starch paper

- In high-quality printing paper, half of the contents are additives, especially stone
- VTT Technical Research Centre of Finland develops ecological starch paper, in which mineral raw materials are replaced with starch
- Starch-based paper can be fully recycled, and finally burned for energy, without detrimental residues
 - recycling processes become cleanier
 - carbohydrates burn more efficiently than stone
- Furthermore, its printing quality is better
- Regarding commercial practice, costs of starch paper should still be lowered

www.vtt.fi/uutta/2006/20051229.jsp?lang=en

Nature herself tests silencing native genes

- Silencing any native gene in a crop species has been tested in millions of times during millennia by the Nature – without ecological problems
- Disarming a plant does not give selection advantage to it in Nature
 - but the weakling and its progeny disappear more rapidly from natural ecosystems
- Accordingly, the silenced gene
- neither conquers ecosystems
- nor causes permanent changes, but
 - at most transient impacts comparable to, or smaller than, the changes resulting from everyday choices of a crop to be cultivated or methods of its cultivation

Genetically modified weaklings?



- Long-term eclogical studies in UK showed that all GM plants under study (oilseed rape, potato, corn, sugar beet)
 - proved weaker in Nature, and
 - disappeared more rapidly than their conventionally bred counterparts

^C Crawley et al. (2001). *Nature* 409: 682-683.

8. Better trees for bio-economy

- Trees utilized by man (fruit trees excluded) have been left out in the cold for millennia
- Their breeding just started last century
- Furthermore, their long generation interval (often decades) retards progress with traditional means
- Accordingly, huge potentials still exist for improving their
- > wood and fibre quality
- resistance to pathogens or drought, etc.
- efficiency of biomass production, for the plethora of uses arising in bio-economy
 - efficiency is a core prerequisite for their true sustainability
- In Finland, tree biomass is by far the most important and net efficient raw material for bioenergy

Agricultural development



New prospects with genetic understanding

- Biotechnology can enable rapid advancements in the production of more and better wood
 - tailor-made to better suit the end use, and
 - produced with rapidly growing trees in much shorter rotation time
- ...in order to fulfil the big challenge of conversing "fossil" societies to ones based on renewable resources
- Efficient production of trees in plantations can help conserve the remaining areas of highest biodiversity such as rainforests

Poplar genome read through

Science (15 September 2006) 313: 1596 - 1604

The Genome of Black Cottonwood, *Populus trichocarpa* (Torr. & Gray)

G. A. Tuskan, S. DiFazio, S. Jansson, J. Bohlmann, I. Grigoriev, U. Hellsten, N. Putnam, S. Ralph, S. Rombauts, A. Salamov, J. Schein, L. Sterck, A. Aerts, R. R. Bhalerao, R. P. Bhalerao, D. Blaudez, W. Boerjan, A. Brun, A. Brunner, V. Busov, M. Campbell, J. Carlson, M. Chalot, J. Chapman, G.-L. Chen, D. Cooper, P. M. Coutinho, J. Couturier, S. Covert, Q. Cronk, R. Cunningham, J. Davis, S. Degroeve, A. Déjardin, C. dePamphilis, J. Detter, B. Dirks, I. Dubchak, S. Duplessis, J. Ehlting, B. Ellis, K. Gendler, D. Goodstein, M. Gribskov, J. Grimwood, A. Groover, L. Gunter, B. Hamberger, B. Heinze, Y. Helariutta, B. Henrissat, D. Holligan, R. Holt, W. Huang, N. Islam-Faridi, S. Jones, M. Jones-Rhoades, R. Jorgensen, C. Joshi, J. Kangasjärvi, J. Karlsson, C. Kelleher, R. Kirkpatrick, M. Kirst, A. Kohler, U. Kalluri, F. Larimer, J. Leebens-Mack, J.-C. Leplé, P. Locascio, Y. Lou, S. Lucas, F. Martin, B. Montanini, C. Napoli, D. R. Nelson, C. Nelson, K. Nieminen, O. Nilsson, V. Pereda, G. Peter, R. Philippe, G. Pilate, A. Poliakov, J. Razumovskaya, P. Richardson, C. Rinaldi, K. Ritland, P. Rouzé, D. Ryaboy, J. Schmutz, J. Schrader, B. Segerman, H. Shin, A. Siddiqui, F. Sterky, A. Terry, C.-J. Tsai, E. Uberbacher, P. Unneberg, J. Vahala, K. Wall, S. Wessler, G. Yang, T. Yin, C. Douglas, M. Marra, G. Sandberg, Y. Van de Peer, D. Rokhsar

^{• 110} scientist from 39 institutions

More efficient means with new scientific knowledge

- Generation interval from seed to seed can be shortened with decades in tree breeding
 - if flowering is launched in young seedlings by adjusting the activity of their flowering genes...
 - ...and molecular biology is utilized for selecting genetically best seedlings in the progeny
- Consequently, some breeding methods utilized in agricultural crops can be taken in use in trees
- With the help of gene technology
 - genetic diversity of related plants can more easily and safely be utilized in breeding
 - tailor-made plantation tree genotypes can better be kept apart from each others and wild ones by breeding these for sterility

Birch can be induced to flower very young – at a few months of age

by enhancing the functioning of one of its native genes



Juha Lemmetyinen©

Birch bred for non-flowering

flowering control





Juha Lemmetyinen©

Many breeding traits are ecologically benign

- Quality traits improving the usability of tree 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
 - because such traits might become more common (though not necessarily dominating) in natural plant populations

Breeding narrow-crowned trees for cultivation

- 'Yield index' of agricultural plants has been much improved during breeding
 - utilizable plant parts now constitute a great proportion of the total crop biomass
- Whereas biomass partitioning is still quite poor in cultivated trees
- E.g. thinner branches would mean that greater share of the materials is accumulated in the stem
 - and thereby also wood quality for construction, furniture and veneer is improved
- Gene for thin branches and narrow crown was found in Norway spruce in Finland in the 80s
- Lepistö (1985), Found. For. Tree Breeding Inf.
 1: 1–6
- In test plantations, narrow-crowned spruce uses its growth area more efficiently
 - and could thereby serve for a fibre-tree with a short-term rotation in the Nordic conditions
- Using gene technology, the gene could also be bred to appropriate other plantation tree species for improving their yield index or wood quality

Narrow-crowned "pendula" spruce



Raimo Jaatinen©

... in a test plantation



Raimo Jaatinen©

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