Agrobiotechnology as a source of materials for industrial biotech.

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Modern biotechnology

2006: Nobel Prize for the discovery of RNA interference

2004: FDA approves the first DNA microarray for diagnostic purposes (AmpliChip)

2003: Completion of the sequencing of the human genome

1999: Production of human stem cells in cell culture

1997: An animal is cloned from adult cells (the sheep Dolly)

1994: Approval for a whole food produced through biotechnology (Flavrsavr tomato)

1989: Human gene therapy is attempted successfully

1986: The 1st biotech anti-cancer drug is produced (interferon); genetically engineered plants (tobacco) are grown in field trials

1983: Development of PCR (polymerase chain reaction)

1982: Recombinant human insulin is approved as the first biotech drug; development of a recombinant DNA vaccine (for livestock)

1981: Production of the 1st transgenic animals (mice)

1975: Production of monoclonal antibodies

1973: Cohen & Boyer perform the 1st successful recombinant DNA experiment

1957: Industrial glutamate production by fermentation

1953: Watson & Crick develop the DNA double helix model

1944: Avery, MacLeod & McCarthy show that DNA is the hereditary material for most living organisms

1928: Fleming discovers penicillin

1865: Mendel discovers that genetic traits (in peas) are passed from parents to offspring in a predictable way

1855: Pasteur proposes fermentation is caused by yeast

1663: Description of the cell

Since B.C.: Use of biotechnology to brew beer, leaven bread, produce cheese and ferment wine

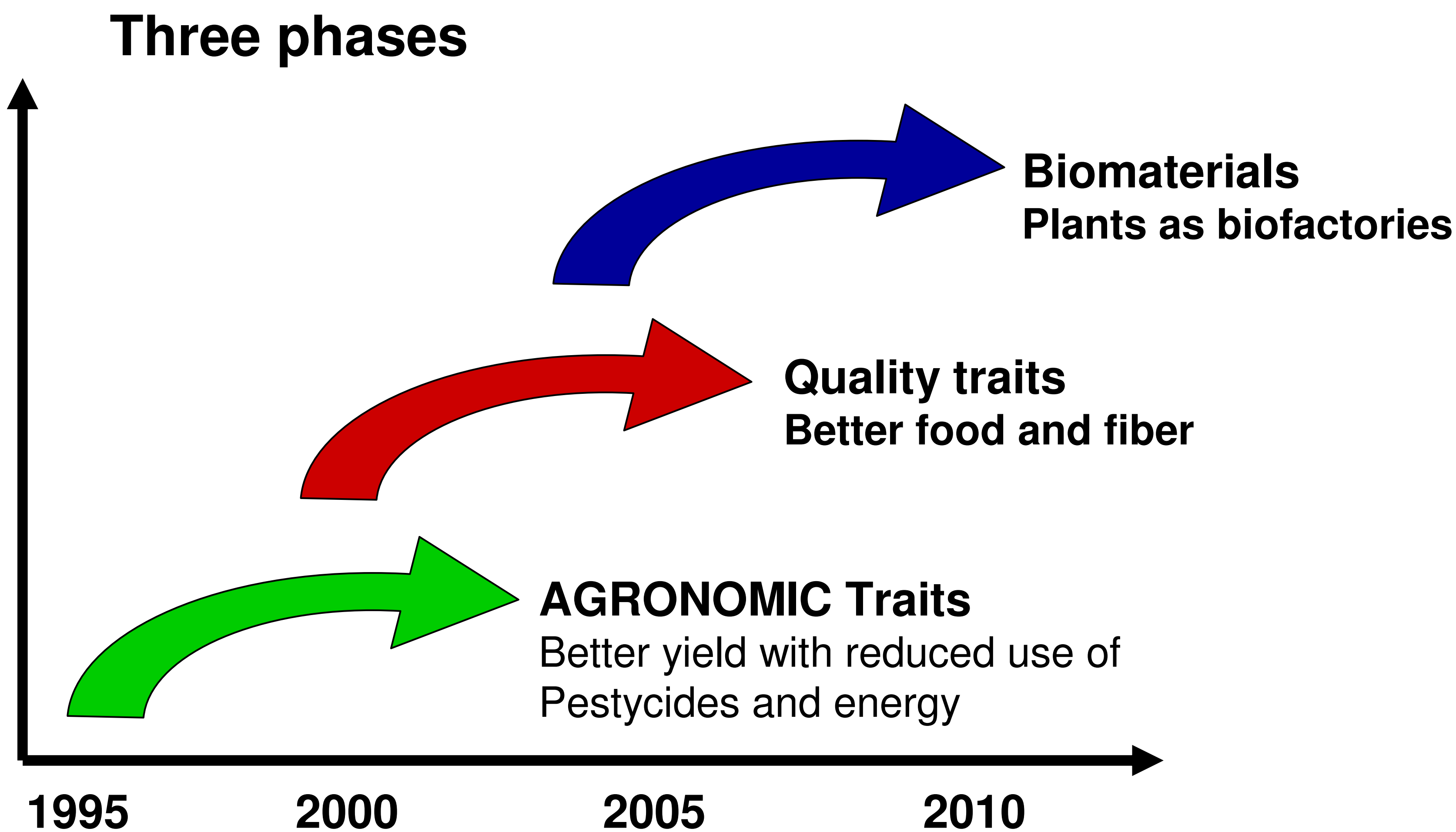
Figure 2-1 Biotechnology milestones

The basic technologies used in modern biotechnology (see Table A.1) are described below.

Table A.1 Technologies used in modern biotechnology

Nucleic-acid (DNA/RNA)-related technologies	• High-throughput sequencing of genome, gene, DNA • DNA synthesis and amplification • Genetic engineering • Anti-sense technology • siRNA technology	Analysis and modification of genetic material
Protein-related technologies	• High-throughput protein/peptide identification, quantification and sequencing • Protein/peptide synthesis • Protein engineering and biocatalysis	Analysis and modification of proteins
Metabolite-related technologies	• High-throughput metabolite identification and quantification • Metabolic pathway engineering	Analysis of metabolites (small molecules)
Cellular-/subcellular-related technologies	• Cell hybridisation/fusion • Tissue engineering • Embryo technology • Stem-cell-related technologies • Gene delivery • Fermentation and downstream processing	Cell manipulation for various applications
Supporting tools	• Bioinformatics	Application of computational tools in analysis and storage of biological data

Trends in agrobiotechnology



MODERN BIOTECHNOLOGY IN PRIMARY PRODUCTION FOR INDUSTRIAL PROCESSES,ENERGY AND ENVIRONMENT

- Industrial production processes
  - Enzyme production
  - Biocatalysis in detergents
  - Biocatalysis in food production
  - Biocatalysis in pulp and paper
  - Biocatalysis in taxtile finishing industry
  - biotechnology in production of chemicals
- Production of biofuel-bioethanol
- Bioremediation
- Emerging biotechnologies in industrial processes and energy

MODERN BIOTECHNOLOGY IN PRIMARY AGRO PRODUCTION

Primary sector in EU – 181 billion euro=2.06% GVA and 5% of employment.

Agriculture – 87%(1.79% GVA)

- Crop production – 50%
- Livestock production – 40%
- Forestry, fisheries – 10%

Biotech methods in crop production

- Molecular markers
- Genetic modification
- Propagation techniques(micropropagation)

Targets for research on non-food plant production

- Develop advanced plant-based raw materials and pharmaceuticals
- Develop plants as energy production systems
- Convert plants into production factories

Develop advanced plant-based raw materials and pharmaceuticals

The development of new plant raw materials and compounds requires the development know-how on key pathways and participating genes, nutrient uptake and transport, energy metabolism, growth conditions, as well as the appropriate enabling technologies.

In five years:

- **Fourty prioritised pathways understood at level of participating genes and products**

- **Efficient molecular gene evolution technology development applicable to genes participating in the aforementioned pathways**

- **Optimise plant recombinant protein expression technology**
- **New transgenic production strategies**

- **New enabling technologies, such as gene replacement and chemical switch technology**

Develop plants as energy production systems

The net gain currently ranges between negative and a factor two compared with input energy. This is insufficient to play a role of importance in resolving future energy demand

In five years

- **Development of production systems (crops,plant cultures, other) with at least 50% lower energy input requirements than current best production systems**

- **Development of options to increase the energy retention of plants by at least fivefold in comparison with today's best performers**

- **Gene replacement technology to optimise selected, high-energy plant biomass production systems**

From:Plants for the Future Technology Platform

Convert plants into production factories

Plants may offer an attractive alternative production system for proteins and other compounds. Their use as a production system depends on their cost, quality, environmental friendliness and the time it takes to produce the compound of interest.

Five years

- **Improved plant gene expression technology for selected non-food plants: mRNA production, translational performance of mRNAs, protein folding, post-translational modification technology.**
- **Compound accumulation and storage technology**
- **Compound transport and secretion technology**
- **New technologies (e.g. gene replacement,transfection technologies and chemical**

  switch) applicable to a range of selected species with the potential to meet commercial performance standards
- **New manufacturing strategies for production,extraction and processing**
- **Development of small-scale manufacturing infrastructure and capacity for nonfood**

  productso Development of plants or plant cells suitable for fermentor-like applications

From:Plants for the Future Technology Platform

PLANT PRIMARY PRODUCTION FOR NON-FOOD USES IN INDUSTRY

- MEDICINAL SUBSTANCES
- BIOFUELS
- BIODEGRADABLE POLYMERS
  - POLYHYDROXYBUTYRATE(PHB)
  - MODYFIED STARCH
- PLANT OILS
  - HIGH OLEIC ACID CANOLA
- WOOD
  - LOW LIGNIN FOR PAPER PRODUCTION

Selection criteria for crops used as industrial resources

- **High yield and agronomy to avoid high production costs**
  (cultivars of crop species will be generally bettet than wilde species).

- **Ease of processability** (to avoid the need of developing new technologies and infrastructure).

- **The utility and value of byproducts.**

- **Identity preservation and out-crossing from non-food crop** (to ensure that industrial products do not enter the food chain).

- **„Fast track" plant breeding and selection methods.**

- **Suitability for genetic engineering**

Genetic features recomended for crops used in industrial processes

(to avoid competition with food/feed production)

- Low input requirements in terms of fertiliser, pesticide and water usage.
- Low impact on biodiversity, soil and water quality
- Efficent land use with high carbon sequestration rate.
- High safety in terms of gene flow.
- Ability to grow on marginal land and those cultivation areas that do not compete with land used for arable food crops.
- Low investments costs in terms of labour, machinery and energy.

APPROXIMATE TIME-LINES FOR DEVELOPMENT OF NEW TRANSGENIC CULTIVAR

1.GENE DISCOVERY	– 3 YEARS
2. TRANSFORMATION, GM PRODUCTION	– 1 YEAR
3.GREENHOUSE TESTING,MOLECULAR CHARACTERISATION	– 1 YEAR
4.PROOF OF CONCEPT IN THE FIELD SELECTION OF BEST VARIANT	– 1YEAR
5.REGULATORY CLEARANCE	- 4 YEARS
6.BREEDING , VARIETY REGISTRATION	– 3 YEARS
TOTAL	13 years

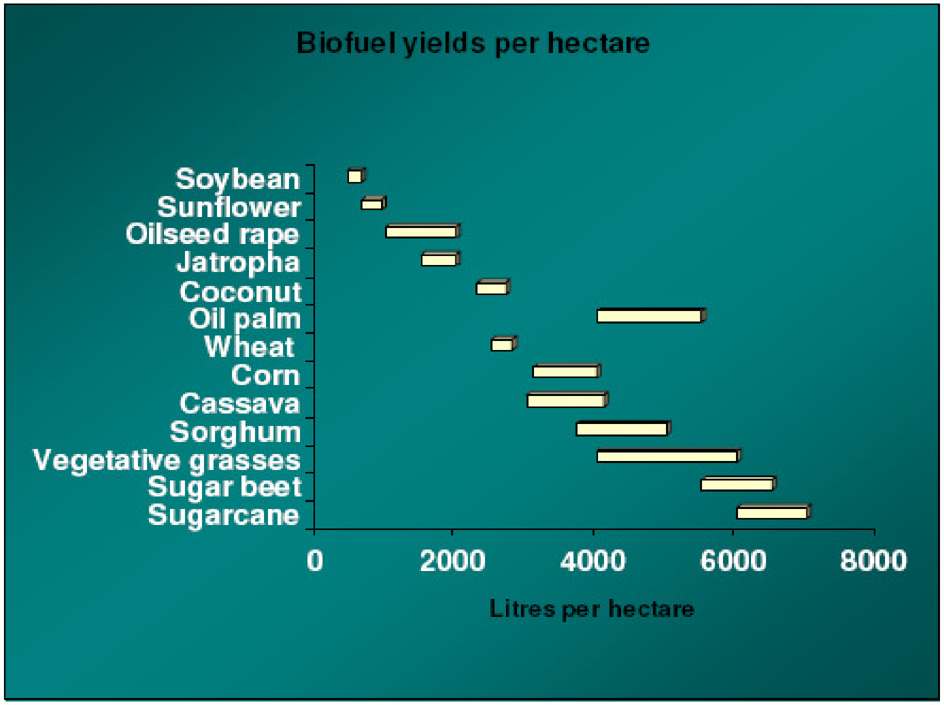
Biotech industrail products aleready available

- Pharmaceuticals under clinical trials(20)
  - Recombinant antibodies
  - Edible vaccines
- Plant derived technical proteins(6)
  - Avidin
  - Tripsin
  - Beta-glucuronidase
  - Lactoferrin
  - lysosime

Examples of biotechnology-based products, production and prices

Product	Annual production(tones	World price(Euro/kg)
Bioethanol	More than 18,4 milion	O,40
Amino-acids		
L-glutamic acid	1 500 000	1,20
L-lysine	700 000	2
L-threonine	10 000	6
L-methionine	400	20
Acids		
Cirtic acid	1 000 000	0,8
Lactic acid	150 000	1,8
Gluconic acid	100 000	1,5
Vitamins		
Vitamin C	80 000	8
Vitamin B12	20	25 000
Riboflavin	30 000	NA
Antibiotic derivatives	2`1 000	NA

Figure 1.2– Estimated per-acre bioethanol yields for various crop types



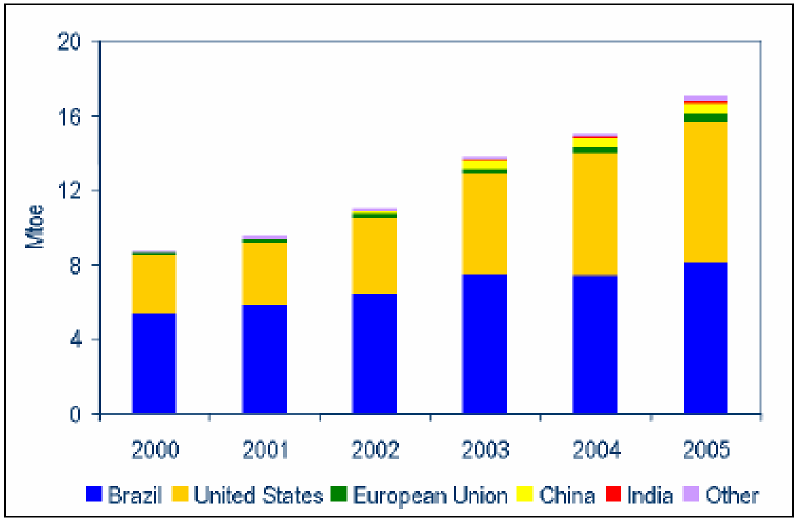
Source: The energy content of biodiesel (~34-36MJ/l) is greater than that of ethanol (~21MJ/l). So readers should not directly compare biodiesel (top half of diagram) with ethanol (bottom half) purely on a l / ha basis

ESTIMATES ON ETHANOL PRODUCTION EFFICIENCY

	Average production efficiency	Fuel process energy efficiency	Well-to wheels GHG emission compared to gasoline
Wheat to ethanol	356 litres/ton of feedstock	O,91 Input:136,5 e.u. Output: 150,oe.u.	Between 19% and 47% reduction Average 32%
Sugar beet to ethanol	86 litres/ton of feedstock	0,67 Input:100,5 e.u. Output: 150,0 e.u.	Between 35% and 56% reduction Average: 46%

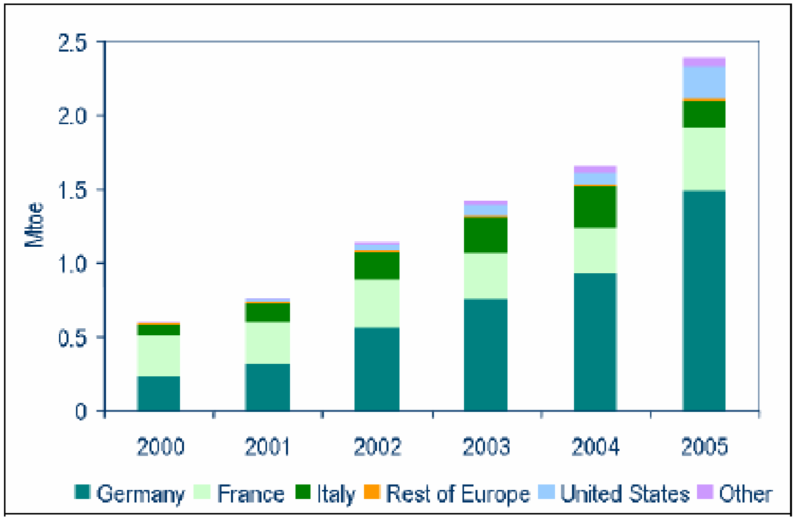
Source: International Energy Agency; e.u.=energy units, GHG=greenhouse gas

Figure 1.3 - Global Bioethanol Production



Source: F.O. Licht, May 09 2007, World Bioethanol and Biofuels Report. Vol.5, No.17

Figure 1.4 – Global Biodiesel Production 2000-2005



Source: F O Licht (2006). World Ethanol Markets: The Outlook to 2015. Special Report No 138, Adapted as Fig 14.3 in IEA World Energy Outlook, 2006

Carbon Capture and Storage: the "Fourth Generation" Biofuels

- "***First generation biofuels"*** use food-based feedstocks (like corn, sugar cane or soybean) as raw material, and utilize processing technologies like fermentation (for ethanol) and trans-esterification (for biodiesel).
  "***Second generation biofuels***" are produced from non-food feedstocks, like lignocellulosic plant biomass (switchgrass, poplar) and non-edible oilseeds (Jatropha) through the conventional method mentioned above and by thermochemical routes (for the production of liquid "synthetic biofuels").
  "***Third generation biofuels***" uses similar production methods on specifically designed or "tailored" bioenergy crops (often by molecular biology techniques) to improve biomass-to-biofuel conversions. An example is the development of "low-lignin" trees, which reduce pre-treatment costs and improve ethanol production, or corn with embedded cellulase enzymes.
- "***Fourth generation biofuels***", are simply a step further from the third generation biofuels. The keywords are "carbon capture and storage (CCS)", both at the level of the feedstock and/or the processing technology. The feedstock is tailored not only to improve the processing efficiency, but it is also designed to capture more carbon dioxide, as the crop grows in cultivation. The processing methods (mainly thermochemical) are also coupled to "carbon capture and storage" technologies which funnels off the carbon dioxide generated into geological formations (geological storage, for example, in exhausted oil fields) or through mineral storage (as carbonates). In this way, fourth generation biofuels are thought to contribute better to reducing GHG (greenhouse gas) emissions, by being more carbon neutral or even carbon negative compared to the other generation biofuels. Fourth generation biofuels epitomize the concept of "Bionergy with Carbon Storage (BECS)"

Introducing a multi-gene metabolic pathway into soybean seeds

Key factors for success:

Multiple promoters needed
Model system that measures product
Promoters tested in metabolic/developmental context
Gene source for each enzyme in pathway
Construct design for expressing multiple genes
Minimum complexity of inserts

Adding an omega-3 lipid pathway pathway to soy

soybean

55% 14%

18:1 → 18:2 —Δ15→ 18:3α

Δ6

18:4γ

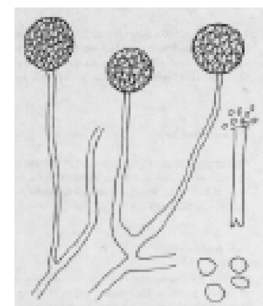
elo 1

20:4

Δ5

20:4 ARA —Δ17→ 20:5 EPA

Challenge:
expressing 5 to 7
new genes in soybean
seeds



Second and third generation GM plants and its products

- Stacked traits (2-3 genes introduced)
- Novel complex traits(e.g. golden rice)
- Molecular farming

Products:

- **Pharmaceuticals**
- **Functional proteins**
- **Industrial enzymes**

Sectors with GHG(CO_2,,CH_4,,N_2O) reduction potential by means of modern biotechnology

- Manufacturing(biotech potential) – 2%
- Industrial combustion - 14%
- Transport - 21%
- Other - 63%