Molecular Biology & Agrobiotechnology

I. 10.000 years of crop plant evolution

II. Global population and nutrition

III. Current topics in plant molecular biology and biotechnology research

Lecture Molecular Biology and Biotechnology of Plants

Rüdiger Hell
Centre for Organismal Studies

Centre for Organismal Studies Heidelberg

Aims of the lecture

I. Fundamentals of modern plant molecular biology

II. Status of plant biotechnology

III. Chances and problems

IV. Economy and ecology

You have to be a good allround biologist to do successful molecular biology!

List Lecture Molecular Biology and Biotechnology WS 2013/14

Prof. Rüdiger Hell (organizer), Centre for Organismal Studies (COS)

Wednesday, 8:15-9:45, COS Lecture Hall, Im Neuenheimer Feld 360

1.	**Wed, 16.10.2013**	**Introduction – Molecular Biology & Agrobiotech (RH)**
2.	**Wed, 24.10.2013**	**Crop Plant Biology (RH)**
3.	**Wed, 30.10.2013**	**Plant Transformation (RH)**
4.	**Wed, 06.11.2013**	**Genomes I (RH)**
5.	**Wed, 13.11.2013**	**Genomes II (RH)**
6.	**Wed, 20.11.2013**	**Gene Expression (RH)**
7.	**Wed, 27.11.2013**	**Resistances (RH)**
8.	**Wed, 04.12.2013**	**Phytopharming (RH)**
9.	**Wed, 11.12.2013**	**GMO safety (RH)**
10.	**Wed, 08.01.2014**	**Tree Biotechnology (S. Strahl)**
11.	**Wed, 15.01.2013**	**Enabling Technologies (H. Hillebrand)**
12.	**Wed, 22.01.2014**	**Improved sink filling & post-harvest physiology (T. Rausch)**
13.	**Wed, 29.01.2014**	**Exam (RH)**

Repetition exam : 10. April 2014 (together with Chemistry)

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I. History of crop plant evolution

- Origin of agriculture
- Domestication (wheat, rice, corn)
- Agricultural technology
- Genetic diversity & biotechnology

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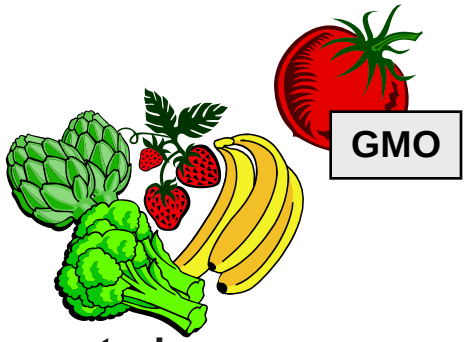
10.000 years of crop plant evolution

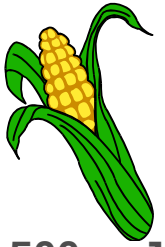
8.000 a. J.-C.

7.200 a. J.-C.



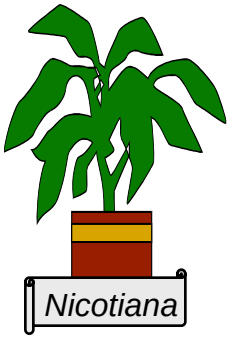
today

6.000 a. J.-C.



4.500 a. J.-C.

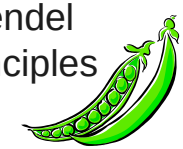
2000: Completion of *Arabidopsis* sequence



1983: Creation of first transgenic plant

1972: Begin of genetic engineering: first cloning experiment
(R. Davis, S. Cohen, W. Arber)

1865: Gregor Mendel discovers the principles of inheritance



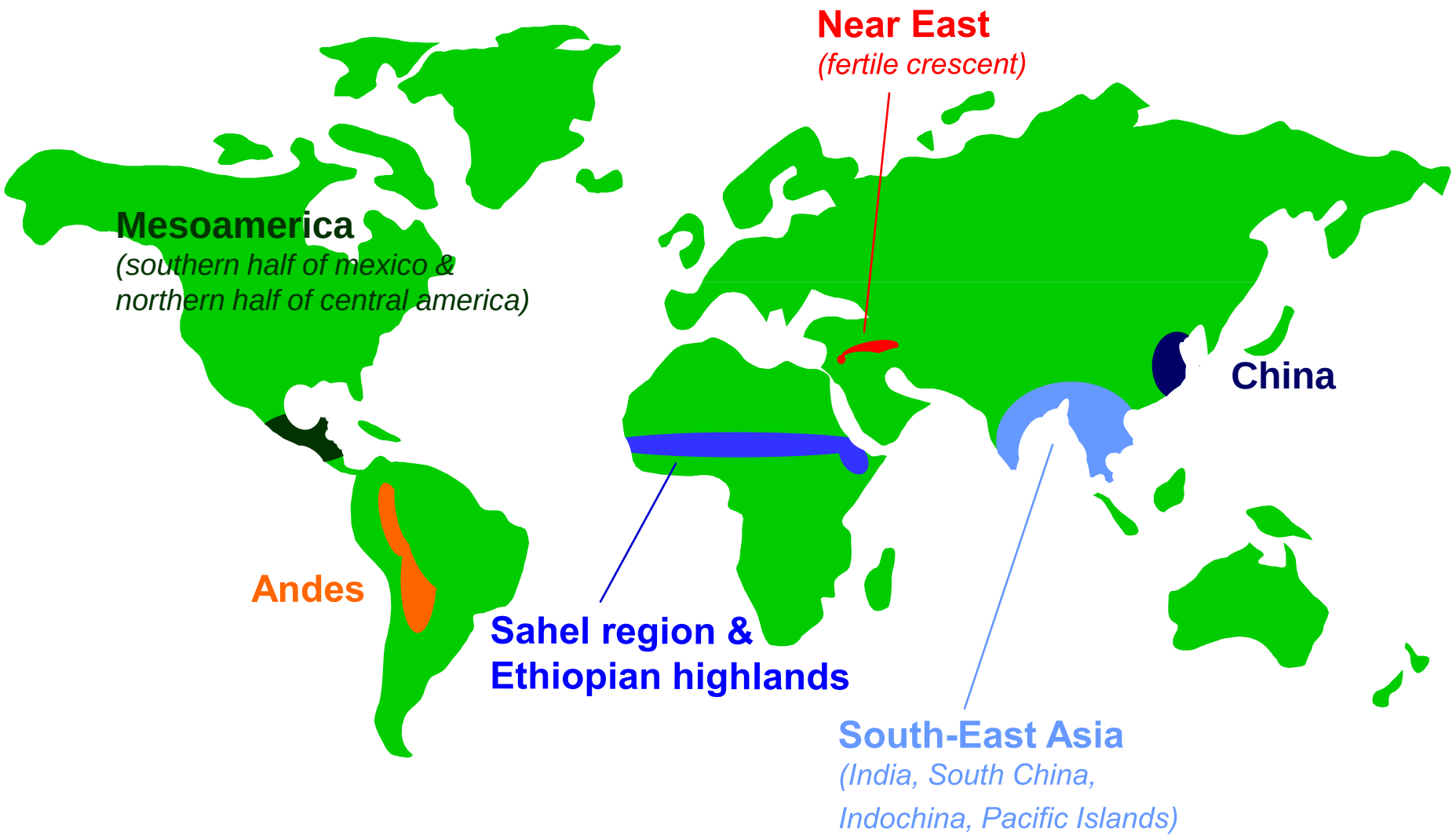
1953: Deciphering DNA (J. Watson, F. Crick, R. Franklin)

The origin of agriculture: Neolithic revolution (8.000 years BC)

- **Global climate changes at the end of the last glacial period**
  - increase in rainfalls: rainy seasons and long hot and dry summers, development of annual plants
  - geographic redistribution of vegetation: imbalance of availability and request for nutrition in some areas of the world
  - population exceeds over local capacity of natural vegetation
  - shortcomings in plant based nutrition urges development of plant management
- **People change from gathering/hunting to farming/breeding**
  - 1 ha supports 10-100 times more people in agriculture than gatherers and hunters
- **Conversion of wild landscapes in fields and grasslands**
- **Domestication of plants and animals**

10.000 years ago agriculture originated at the same time at different locations in all continents

The origin of agriculture (I)



The origin of agriculture (II)

Worldwide distribution of plants and their nutritional functions

Crop types	Central America	South America	Africa	Near East	South-east Asia	China
Cereals	X		X	X	X	X
Pseudo-cereals	X	X				
Grain legumes	X	X	X	X	X	X
Roots & tubers	X	X	X	X	X	X
Oil plants	X	X	X	X	X	X
Fibers	X	X	X		X	
Fruits	X	X	X	X	X	X
Vegetables & spices	X	X	X	X	X	X
Stimulants	X	X	X	X		X

Domestication: Accelerated evolution

	Seed dispersal	Seed dormany	Plant shape	Adaptation to photoperiod	Extremes of shape, colour and proportions	Resistence against pathogens and pests; toxic compounds
Natural environment	-Spontaneous distribution by opening mechanisms	-Prevention of germination at adverse conditions -Allows survival in soil until environment improves	-Competition: Access to light, water, nutrients -Vegetative development equally important as seeds and fruits; low yield	-Controls development until generative phase under sutiable environment -Allows spread of seeds under optimal conditions	-Hiding of reproductive organs after flowering	-Defense mechanisms: Morphology, toxic compound (poppy: morphin; Digitalis/Fingerhut; Cyanogenic glucoside/Cassava -Fruits, seeds in warning colour
Agro-ecosystem	-Seeds remain with plant	-Less competition and more predictable conditions -Simultaneous germination	-Compact architecture to reduce competition -Less branches and shorter secondary shoots	-Loss of photoperiodicity allows several generations and harvests per year	-Enormous diversification of forms and colours -Enforced by human selection	- Plant protection by humans - Selection of non-toxic varieties -Selection for special processing methods





Domestication syndrome

- Similar traits/properties are chosen to differentiate crop plants from wild ancestors
  - involvement of only few genes
  - simple inheritage by restricted genomic localization
  - trait gene expression independent from environment

- Final result:
  - crop plants depend on cultivation by man (sowing & harvesting)
  - man depends *vice versa* on sufficient yield production

Synchronization in the field *vs*.
adaptation towards multiple influences in a natural environment

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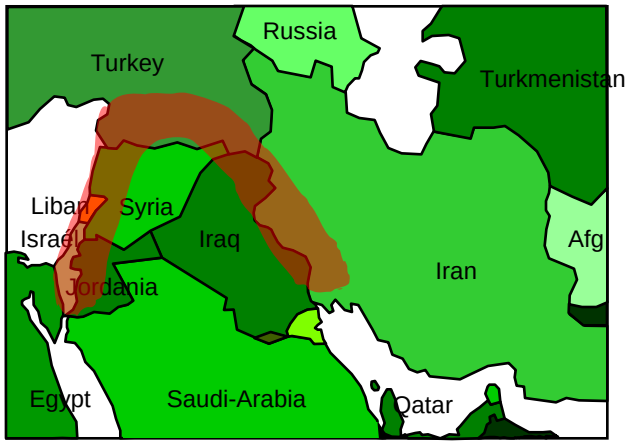
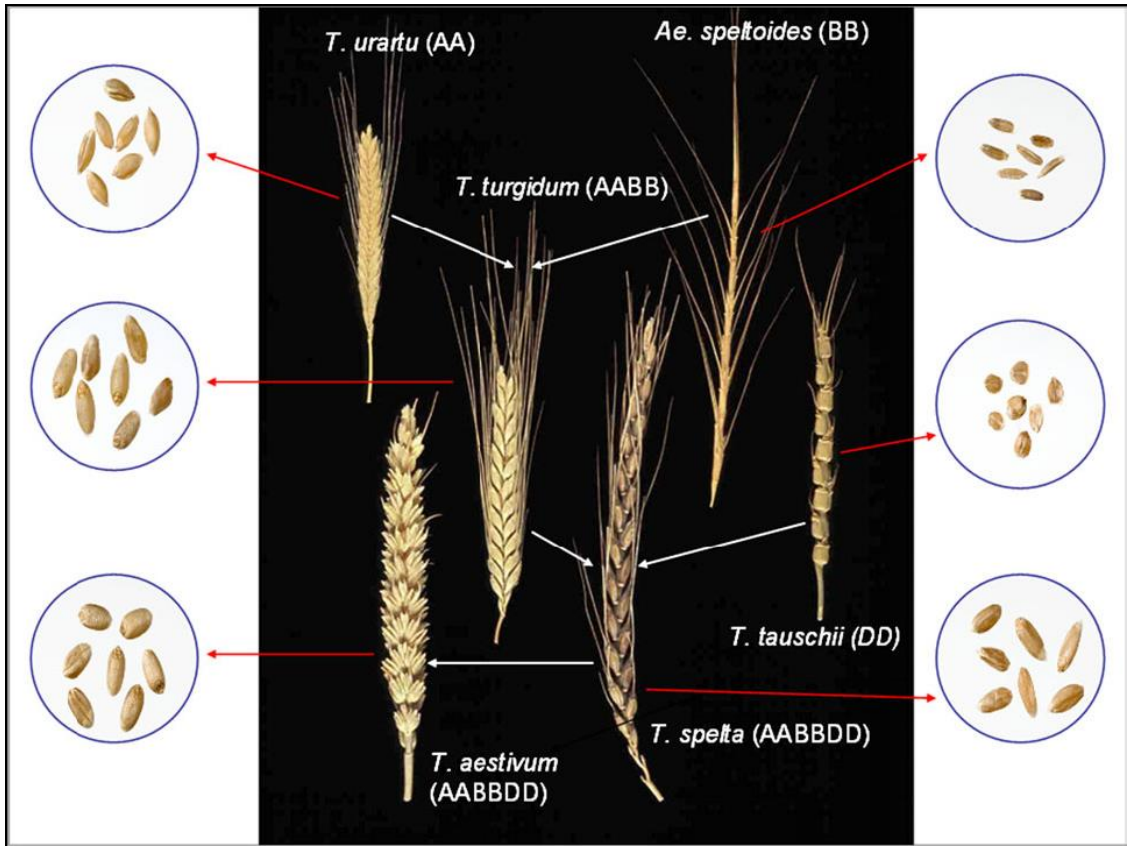
Wheat was domesticated in the Near East

- Enormous economic importance
  Foodstuff for 54% of world population; feedstuff
- Planted on >215 Mio. ha
  on 5 continents
- Total production: ca. 600 Mio. t in 2001
- Wheat: Collective term for the cereal *Triticum*
  - *Triticum aestivum* - (Bread wheat)
  - *Triticum durum* - (Pasta wheat)
  - *Triticum monococcum* – (Einkorn)
  - 3000 recent forms



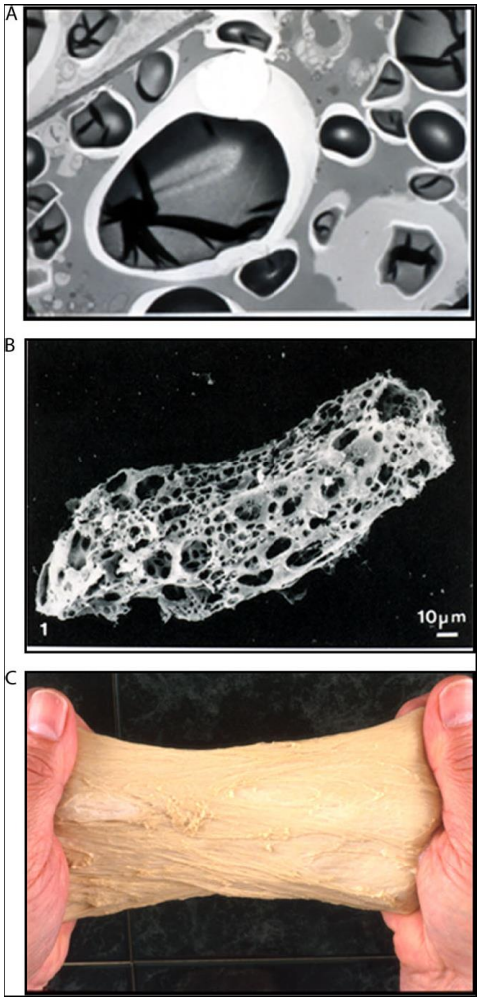
- Most of the species of the family originate in the Near East
  - Species of the Genus *Triticum* und *Aegilops*
  - Fertile Crescent: Turkey, Libanon, Israël, Jordan, Syria, Iraq, Iran
  - 2 essential genetic events determine the evolution of wheat:
    - Normal evolution (Mutations) und chromosome divergence led to different forms (A, B, D) of the 7 chromosomes
    - Spontaneous crossing of divergent parents generated polyploid forms: Tetraploids (4x7) und Hexaploids (6x7)
  - Doubling of chromosomes prevents sterility of hybrids

Domesticated wheat is derived from three wild parents



From: Shewry, P.R. (JEB, 2009)

Fused protein bodies in the endosperm



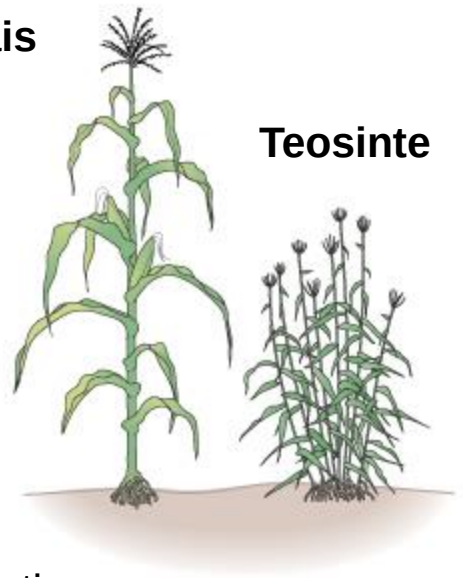
Dough: a continuous proteinaceous matrix

Rice was domesticated independently in Africa and Asia

- 34% of world population depends on nutrition by rice
- Cultivated area of ca. >150 Mio ha (China and India)
- Worldwide production: 600 Mio t in 2001
- Origin in Asia: *Oryza sativa* (derived from *O. nivara*)
  - 2 wild forms dominate in South-east Asia:
    - → *Oryza nivara* (annual)
    - → *Oryza rufipogon* (perennial)
  - 5000 recent forms
- Origin in Africa: *O. glaberrina* (derived from *O. barthii*)
  - Resistent against adverse climates
  - Extensive cultivation and spontaneous disperal of seed
- Particular aspects of rice plantation
  - Oldest propargation areas 8000 years old (Yangtze, China)
  - Domestication of *O. nivara* was followed by divergence into 2 subforms *indica* und *japonica*:
    - → indica: non-sticky; long kernels, tropical climate, deep-water rice
    - → japonica: sticky; short kernels, moderate climate, dry rice

Maize was domesticated in Central and South America

- Provides 12% of world population with food
- Main cultivation areas:
  - USA, Brasil, Mexico, India, France, Italy
- Technical applications
  - feed stuff
  - Industrial ethanol production
- Total production: 600 mio t in 2001



- Original species: Téosinte
  - Geographical distribution corresponds to old american civilazations
  - 6000 recent forms
- Single domestication event in mountains of West-Mexico
- Simultaneous flowering of Teosinte and Maize allowed crosses
- World-wide distribution of maize from 1492 on
  - Introduction of ‘Old World’ species (wheat, rice) into ‘New World’
  - Introduction of Maize, Tomato, Potato into ‘Old World’

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Agricultural technology (ca. 1850 – 1950)

What are the changes?

- Inventions of new machines since 1850
- Inorganic fertilization (J. Liebig, 1855)
  - Application of chemicals to replace natural fertilizers
- New and improved crop plant species (Mendelian Laws, 1910)
  - genetic breeding
- Plant protection measures (since 1950ies)
  - Application of herbicides and pesticides
- Green revolution (N. Borlaug, since 1960ies)
  - Breeding of resistance traits, short (dwarf) cereals
- Irrigation technology
  - 18% of agricultural surfaces yield 40% of total production
  - water is the main limitation for agricultural production of the 21st century
- Information technology
  - precision agriculture (computers, satellites, GPS)

18% of agricultural surfaces yield up to 40% of total production

Results of agricultural technology in developing countries

Country	Plant	Yield in t ^{3}/ha	
		1963	1983
India	wheat	0,9	1,7
	rice	0,9	2,2
China	wheat	1,0	2,5
	rice	2,0	4,7

(FDA, 1989)

Norman E. Borlaug (1914-2009)

Improvement programs by CGIAR Institutes (**C**onsultativ **G**roup for **I**nternational **A**gricultural **R**esearch (founded in 1971 by world bank)

- ICRISAT; India - drought resistance
- CIAT; Colombia - insect resistance in beans
- IRRI (International Rice Research Institute); Philippines - rice
- CIMMYT (International Center for the improvement of maize and wheat) in Mexico

The green revolution resulted in yield duplication.

Results agricultural technology: Limited crop plant diversity today

Culture	Number of varieties	Major varieties	
		Number	Cultivation area (%)
Beans - dry	25	2	60
Beans - lose	70	3	76
Cotton	50	3	78
Maize	197	6	71
Peanut	15	9	95
Soybean	62	6	56
Wheat	269	9	50

National Research Council, 1972

The limitation in crop plant diversity is a result of the cultivation of few varieties in huge dimensions.

Creation of genetic diversity

- conservation programs for natural diversity
- application of genetic ressources for cultivation programs
  - african rice has elevated drought resistance phentotypes
  - central american beans show a better adaptation to P-depleted soils
  - ...
- Mutant production
  - spontaneous mutations (Ruby Red, 1926)
  - irradiation mutations (Rio Red, 1988)

- Production of Hybrids and polyploids
  - plants are much more promiscuous than animals!
  - ‘ Modern’ strawberrys
  - Triticale
- Genetic engineering: *‘ genetic revolution ’*

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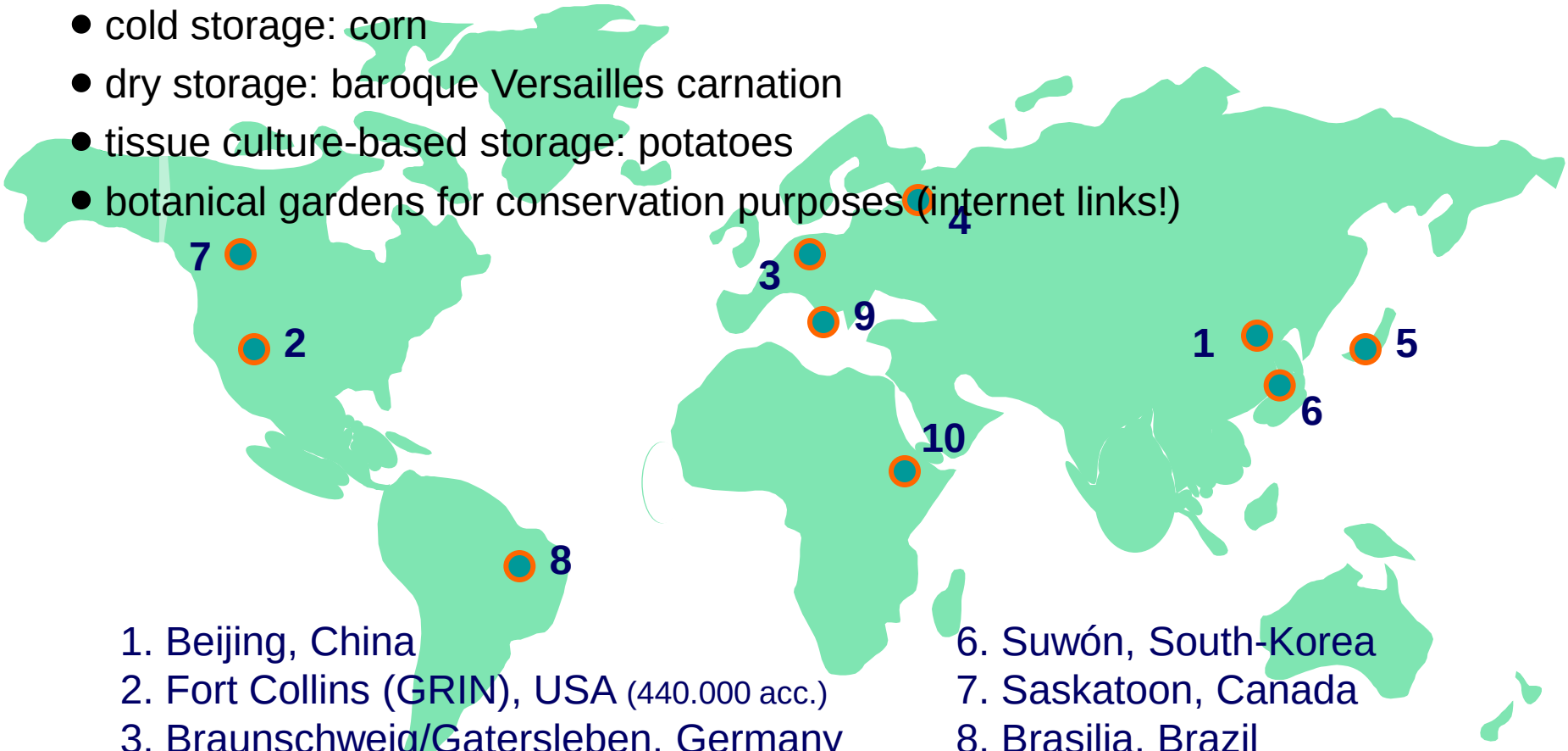
Preservation of genetic diversity

Maintaining varieties in gene bank depositions worldwide

- cold storage: corn
- dry storage: baroque Versailles carnation
- tissue culture-based storage: potatoes
- botanical gardens for conservation purposes (internet links!)



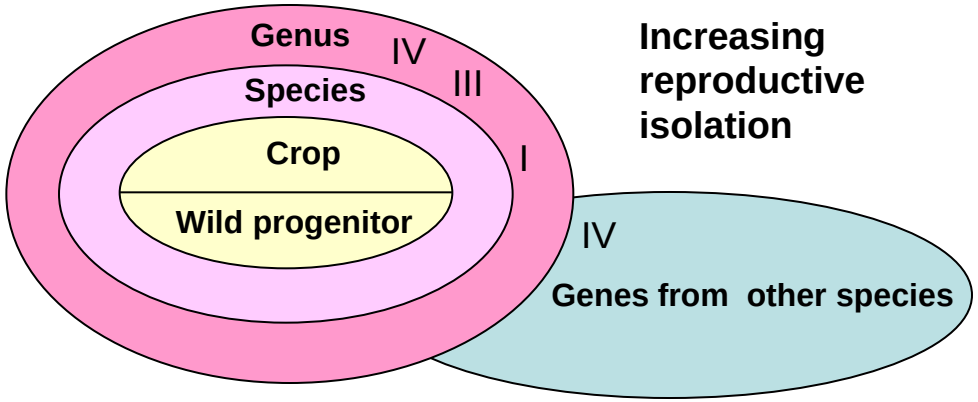
1. Beijing, China
2. Fort Collins (GRIN), USA (440.000 acc.)
3. Braunschweig/Gatersleben, Germany
4. St. Petersburg, Russia
5. Tsukuba, Japan
6. Suwón, South-Korea
7. Saskatoon, Canada
8. Brasilia, Brazil
9. Bari, Italy
10. Addis Abeba, Ethiopia

http://www.ipk-gatersleben.de/en/02/02/

Access to genetic material

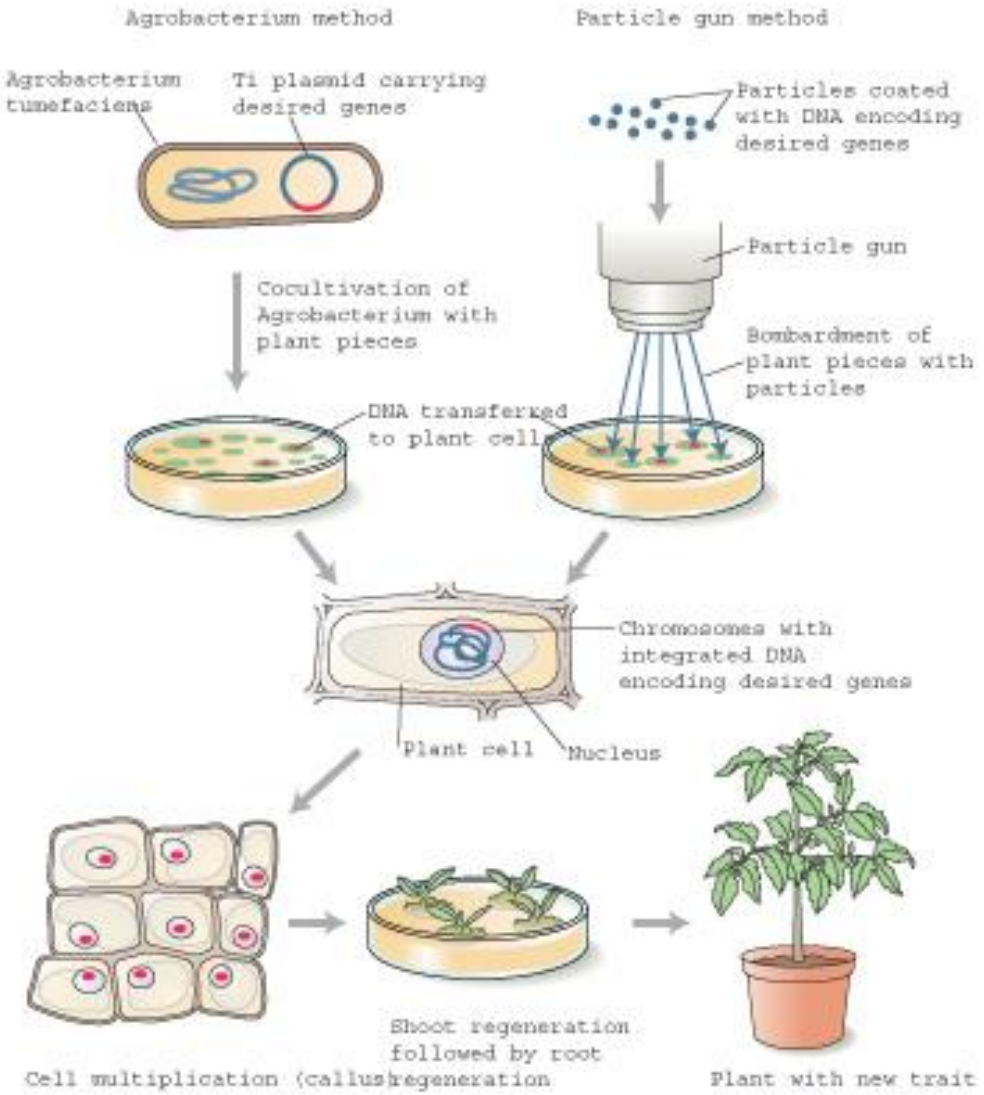
- Species definition: all individuals can cross
- Classes of genetic ressources:

Class I - biological definition of species (crop plant + wild plant)
Class II - forced crosses (low frequency - example: strawberry)
Class III - crosses only by enhanced cultivation technology (example: Triticale)
Class IV - plant species *vs*. species from other kingdoms



The genetic revolution allows to overcome the borders between organisms.

Production of transgenic plants



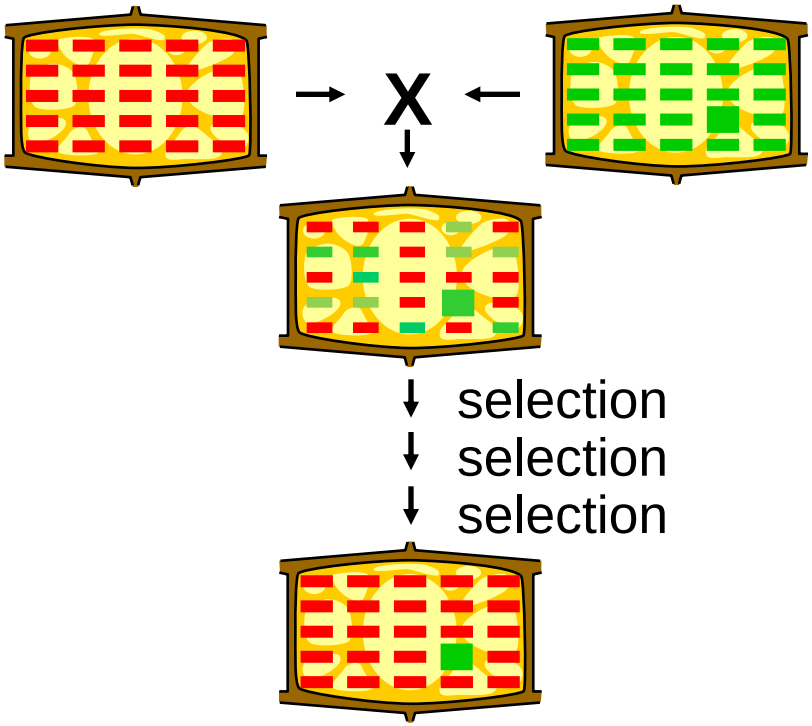
- Abbreviation of conventional breeding
- Allows for integration of foreign genes into a plant genome

Abbreviation of breeding by biotechnology

Breeding

Crop Plant

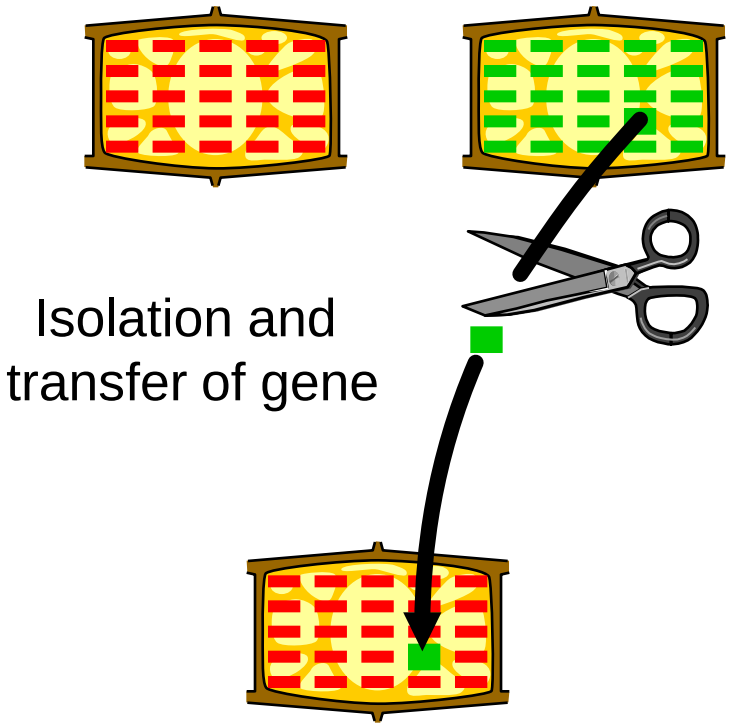
Wild Plant



Genetic Engineering

Crop Plant

Wild Plant



Biotechnology allows for a controlled and directed improvement of a crop plant ***and*** to overcome barriers between species/organisms

Molecular Biology & Agrobiotechnology

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- Requirement and distribution of arable land
- Cultivation areas of transgenic crops

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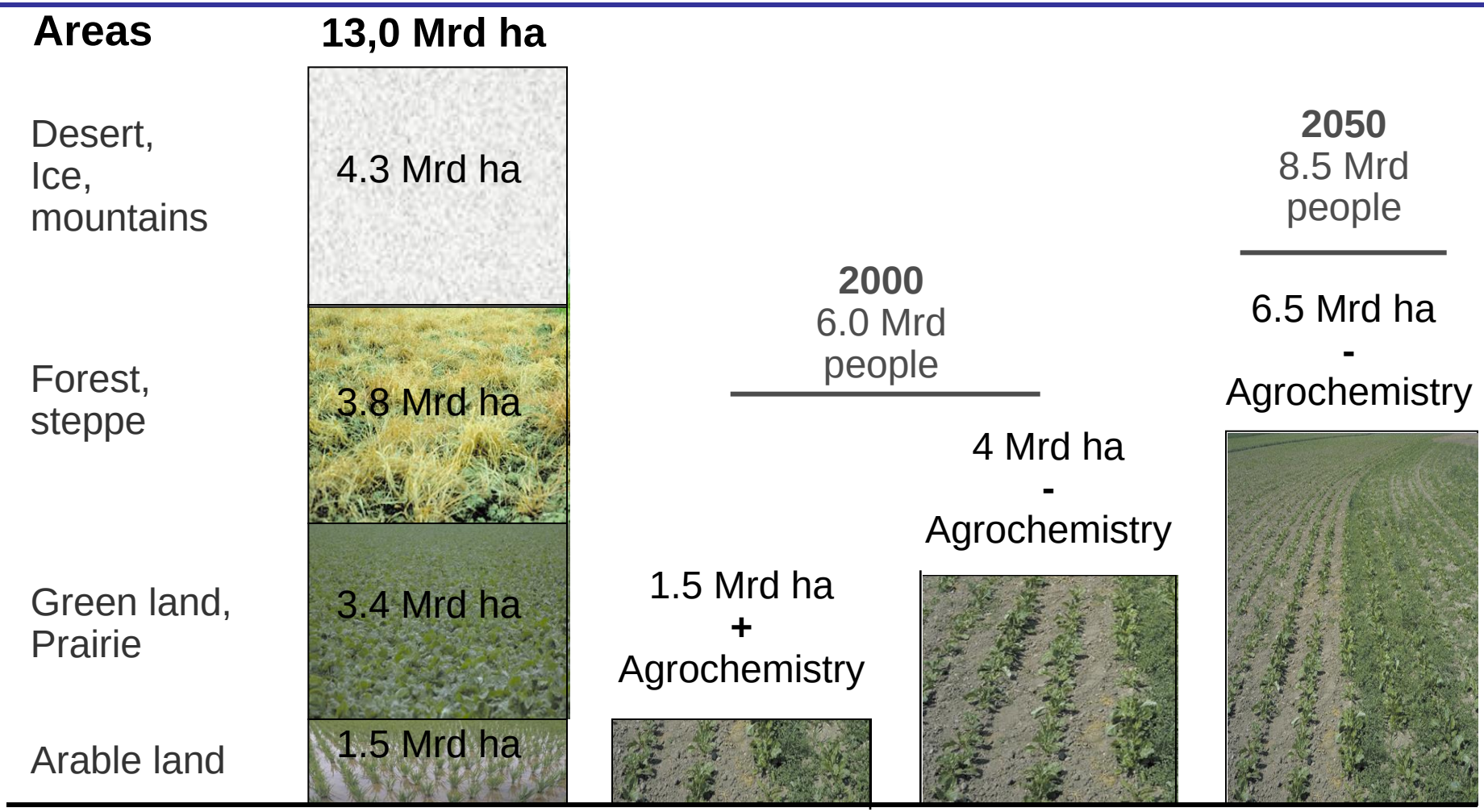
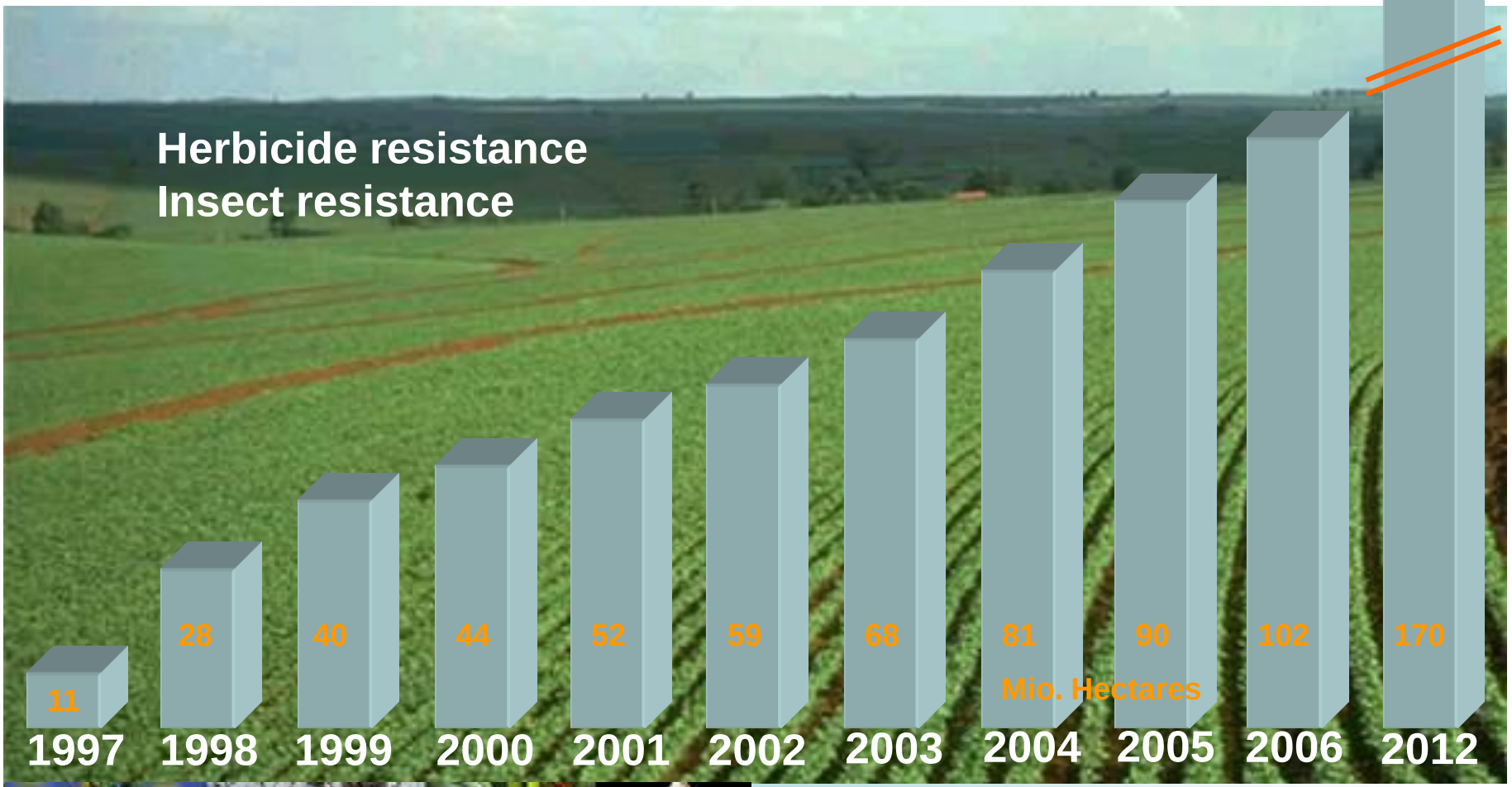
Production origin of plant food stuff



Requirement for arable land will exceed available area in 2050

Source: D.T. Avery, US-Hudson Institute, Deutsche Stiftung Weltbevölkerung 1998

Cultivated area of transgenic plants 1997 - 2010

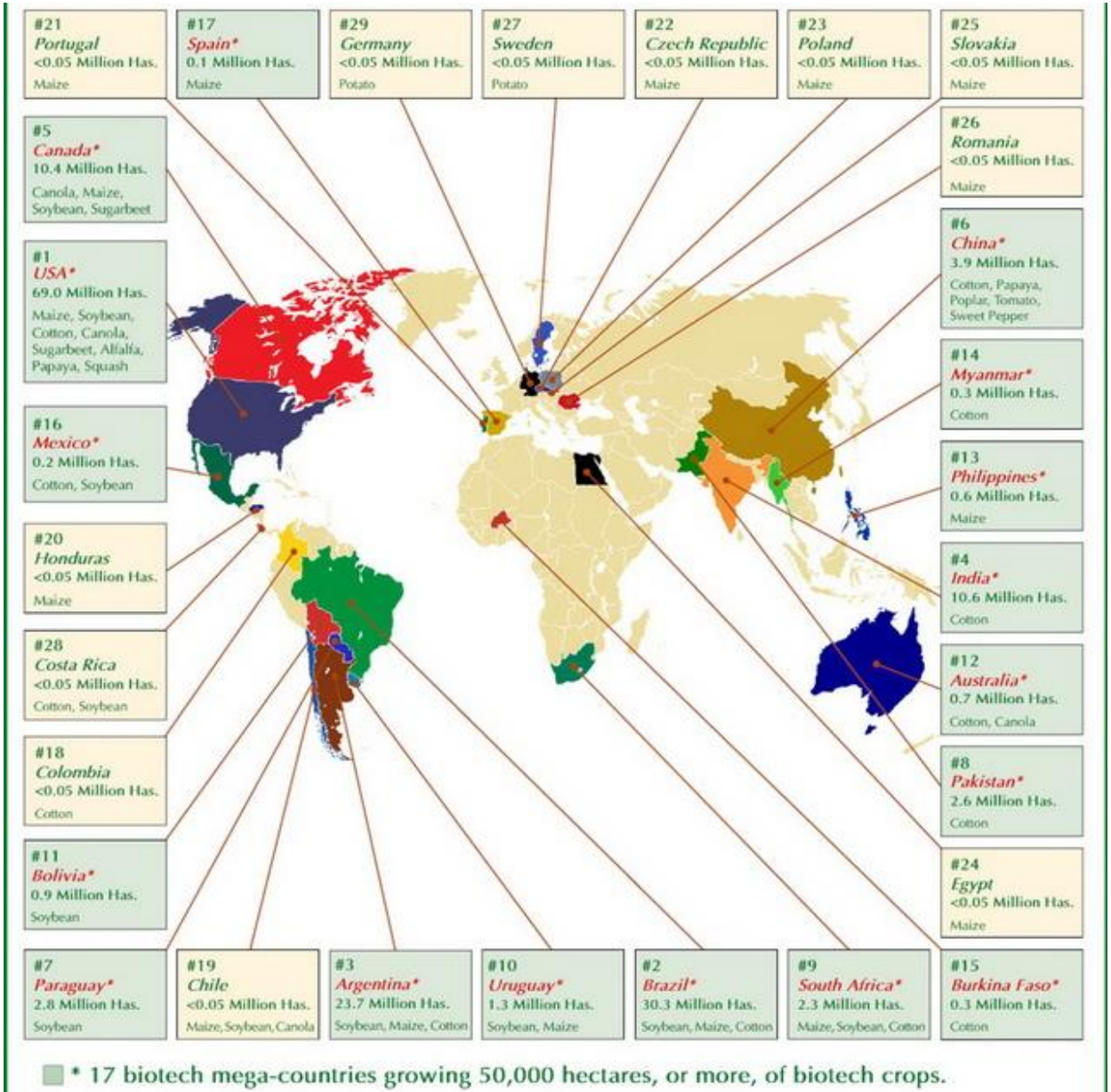


Rape seed
Cotton
Maize
Soybean

Germany‘s total area: 36 Mio. ha

From: C. James, ISAAA Executive Brief 44-2012

Biotech crop countries 2011



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- Identification of trait genes
- Input and output traits
- The next generation of crop plants

Gene identification: Genes und Genomes

7382 Complete Genome Projects on 15. Oct. 2013

Archaeal: 234 Bacterial: 6837 Eukaryal: 311

- **Procaryotes**: Archaea (*Pyrococcus, Halobacter*, ...), Eubacteria (*Clostridium, Bacillus, Escherichia* (4.100kb), *Bradyrhizobium, Vibrio*, ...)

Incomplete projects: 24.445

Species	Name	Genome size (kb)	# ORFs
A. gambiae	Malaria mosquito	278.000	14.000
A. thaliana	Thale cress	115.428	25.498
C. elegans	Nematode	97.000	19.099
D. melanogaster	Fruitfly	137.000	14.100
H. sapiens	Man	~3 x 10^6 kb	~ 30.000
M. musculus	Mouse	~2.5 x 10^6 kb	~ 30.000
O. sativa indica	Rice	420.000	50.000
S. cerevisiae	Baker‘s yeast	12.069	6.294

http://www.genomesonline.org/

Classification of transgenic traits

Input traits
- Agricultural properties
- Producer-oriented
- 1. Generation of transgenics

Output traits
- Quality properties
- Consumer-oriented
- 2. Generation of transgenics

Molecular biology goals towards input traits

Input traits (agricultural properties)

- Improved germination
- Improved performance
  - Abiotic stress resistence
    - → Low temperatures
    - → High temperatures
    - → Drought
    - → Salt tolerance
    - → Herbicides
    - → .....
  - Biotic stress resistence
    - → Virus
    - → Bacteria
    - → Fungi
    - → Nematodes
    - → Insects
    - → ...

Input traits: Herbicide resistance

Total herbicides

- Glyphosate (RoundupReady, Monsanto)
  Resistance gene origin: *Agrobacterium umefaciens*
  Target: EPSP Synthase in Chloroplasts

- Phosphinotricine (Liberty Link, Aventis)
  Resistance gene origin: *Streptomyces hygroscopicus*
  Target: Glutamine Synthetase in Chloroplasts

***RoundUp Ready* rape seed**

Before **After**

Input traits: Resistance against abiotic stress

Nature provides templates for tolerance mechanisms:

Ice plant
(Mesembryanthemum)

- Drought
- Salt

Moss
(*Physcomitrella patens*)

- Low temperature
- Salt

Cactus

- Drought

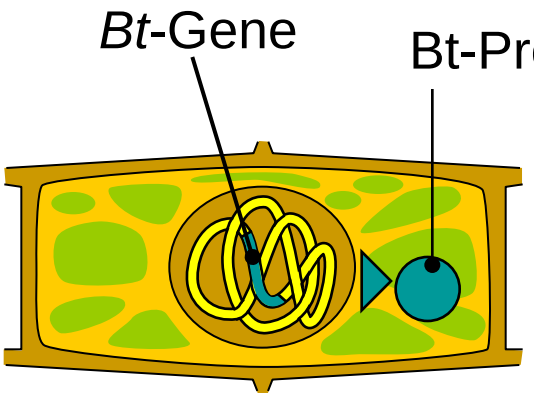
Resurrection plant
(Craterostigma)

- Drought

Input traits: Resistance against biotic stress (I)

Resistence against insects: Bt maize

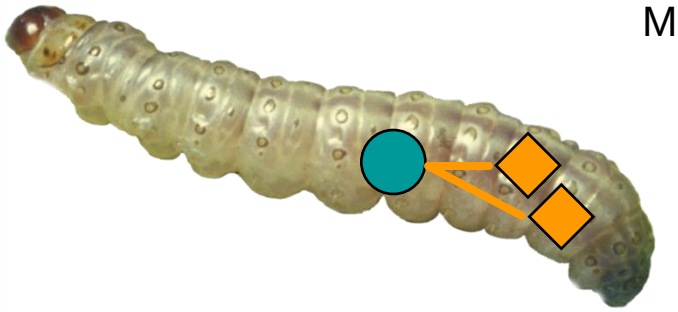
- **Origin: *Bacillus thuringiensis***



Expression of *Bt*-Gene:
Production of Bt-Protein

Corn borer (Maiszünsler)

Main pest in corn production



Enzymatic hydrolysis of Bt-Protein:
Toxic fragments are lethal

Classification of transgenic traits

Output traits (quality properties)

- Higher yield
- Production of plant components
  - Nutritional value
  - Nutritional quality
  - Nutraceuticals
  - Production of pharmaceuticals
  - Production of animal feed
  - Industrial enzymes
  - Plastic
  - Polymers
  - ...

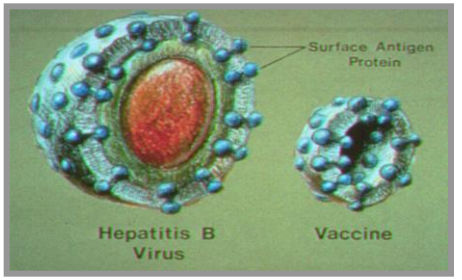
Output traits: Improved nutritional quality

‘Golden Rice’ – Rice with more provitamine A

- Project of Ingo Potrykus, ETH Zürich
- 50% of world population is fed by rice
- Rice kernel without husk has no vitamine A
- Vitamine A deficiency affects betrifft 800 million people (eyes, immuno defense, blood formation, fertility. High mortality rate among children)
- Scientific approach: Introduction of all required genes for synthesis of β-Carotin
  - Phytoene Synthase *(Narcissus)*
  - Phytoene Desaturase *(Erwinia)*
  - Lycopine Cyclase *(Narcissus)*
- Other genes of interest:
  - Ferritin for enhance iron content
  - Essential amino acids (methionine)

Next generation of output traits: nutraceuticals and molecular pharming

- Antibodies: ‘Plantibodies’
  Recombinant IgGs, single chain F_{ab}
- Vaccines: Immunization
  Lyme disease, caries, viruses
- Therapeutic proteins:
  Protease inhibitors, hirudin, somatotropin, interferon, enkephalin
- Nutrient additives:
  Vitamin A, iron, tocopherol, essential amino acids



Summary

- **The development of crop plants has multiple origins and accompagnied the evolution of modern humans**
- **The historical sequence of events resulted in a concentration of few species with a limited gene pool**
- **Strong selection pressure led to the depence of crop plants from man**
- **Drastic genetic shifts by crosses and mutation caused an accelerated evolution to crop plants**
- **The successful increase of food production by breeding can not compensate the increase in population and loss of arable land in the long run**
- **Biotechnology allows the renewal of genetic diversity of crop species and the introduction of new traints**
- **The 1st generation of transgenic plants refers largely to input traits : Herbicide resistence (Round-up Ready) and insect resistence (Bt-toxin)**
- **The 2st generation of transgenic plants will be gentically more advanced and in addition to increased yield will provide new and better products**

Next lecture: Crop Plant Biology

Global area of biotech crops by country 2011

Rank	Country	Area (million hectares)	Biotech Crops
1	USA*	69.0	Maize, soybean, cotton, canola, sugarbeet, alfalfa, papaya, squash
2	Brazil*	30.3	Soybean, maize, cotton
3	Argentina*	23.7	Soybean, maize, cotton
4	India*	10.6	Cotton
5	Canada*	10.4	Canola, maize, soybean, sugarbeet
6	China*	3.9	Cotton, papaya, poplar, tomato, sweet pepper
7	Paraguay*	2.8	Soybean
8	Pakistan *	2.6	Cotton
9	South Africa*	2.3	Maize, soybean, cotton
10	Uruguay*	1.3	Soybean, maize
11	Bolivia*	0.9	Soybean
12	Australia*	0.7	Cotton, canola
13	Philippines*	0.6	Maize
14	Myanmar*	0.3	Cotton
15	Burkina Faso*	0.3	Cotton
16	Mexico*	0.2	Cotton, soybean
17	Spain*	0.1	Maize
18	Colombia	<0.1	Cotton
19	Chile	<0.1	Maize, soybean, canola
20	Honduras	<0.1	Maize
21	Portugal	<0.1	Maize
22	Czech Republic	<0.1	Maize
23	Poland	<0.1	Maize
24	Egypt	<0.1	Maize
25	Slovakia	<0.1	Maize
26	Romania	<0.1	Maize
27	Sweden	<0.1	Potato
28	Costa Rica	<0.1	Cotton, soybean
29	Germany	<0.1	Potato

From: C. James, ISAAA Report on Biotech Crops Feb. 2012