From Genes to Transgenic Plants
3503-450

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https://www.uni-hohenheim.de/biotechnologie
Password: MB2013
Program

Fridays 10 - 12h; 13 -15 HS 10; Field trip 28.06.2013 (Tentative date)

12.04.: Introduction to cell biology & plant tissue culture (Gerd Weber)
12.04.: Gernes & genomes (Gerd Weber)
19.04.: Sequencing (Gerd Weber)
19.05.: Bioinformatics (Gerd Weber)
26.04.: Introduction to plant transformation 1 (Robert Boehm, Gerd Weber)
26.04.: Plant transformation (Robert Boehm, Gerd Weber)
03.05.: Transgenic plants (Robert Boehm, Gerd Weber)
10.05.: Forward and reverse approaches for gene isolation (Uwe Ludewig)
17.05.: Mutations and mutant pools of mutant lines (Uwe Ludewig)
07.06.: "Omics"- technologies (Uwe Ludewig)
14.06.: Molecular biology of tissue differentiation (Götz Reustle)
14.06.: Gene silencing (Götz Reustle)
28.06.: Field trip to Ornamental Bioscience (Robert Boehm) 14-16h
Introduction to cell biology and tissue culture

Gerd Weber
Plant Breeding and Biotechnology
University of Hohenheim, Stuttgart
10,000 Years of Biotechnology

30 years of gene technology: transgenic bacteria, fungi, animals, plants
Recently: Genomic sequences
Definition of Plant Biotechnology

In a broad sense:
Plant biotechnology covers many of the tools and techniques that are commonplace in agriculture and food production.

In a narrow sense:
Biotechnology considers only the new DNA techniques, molecular biology and reproductive technological applications, like gene manipulation, gene transfer, DNA genotyping and cloning.

FAO Statement on Biotechnology
Plant Culture *in vitro* = „in glass”
In Vitro Culture: Clonal propagation and disease-free plants

Potato reproduction by stem tubers
Ornamentals (colour morphs, mutants)
Strawberry meristem culture (generating virus-free plants)
Rooting of cuttings (conifers)
Grafting of trees (plum, apple): circumvent seed dormancy
Hop
Coniferous trees
Banana
Citrus
Transgenics
Reasons for *in vitro* cultivation of plant material

Controlled environmental conditions
- science, asymbiotic orchid seed culture, metabolite production

Pathogen-free material
- once pathogen-free, the material propagated under sterile conditions remains pathogen-free

Multiplication
- rapid clonal propagation; also done ex vitro for many plants
- Embryo rescue (infertile hybrids)

Cryopreservation
- Propagation and Distribution of Mutants, Colourmorphs, etc.
- possible also for *ex vitro* clonally propagated plants

Genetic engineering
- true regeneration necessary, most meristems are not transformable
In Vitro pollination

*Petunia hybrida*
Propagation of hybrids

*Asparagus spec. L.*
Interspecies crossings

*Torenia* spec. (Scrophulariaceae; snapdragon family)
Wishbone Flower
Mutation – new varieties

*Kohleria* spec. (Gesneriaceae)
Industrial–scale production in plant cell cultures

Shikonin from *Lithospermum erythrorhizon* (Boraginaceae)
African oil palm: Costa Rican Dwarf Variety
In Vitro propagation of dwarf oil palm in Costa Rica
In Vitro propagation of oil palms

Palmatica, CR
Pathway of plant transformation

1. source

2. DNA isolation

3. cloning of genes

4. gene constructs

5. transformation & tissue culture

6. plant breeding
Genetic engineering of carnation

Petal color
“Moon Series” carnations produce a new anthocyanin

Delphinidin

FLORIGENE Moonaquamarine™
FLORIGENE Moonlust™
FLORIGENE Moonlite™
FLORIGENE Moonshade™
FLORIGENE Moonshadow™
FLORIGENE Moonvista™
Definition of plant tissue cultures?

„In plant tissue cultures, sterile plant material is cultured under aseptical conditions in usually defined sterile culture medium often solidified by agar”

(Heß, 1992, p. 15)
Which are the requirements for *in vitro* culture?

- Conditions for working in a sterile environment
- Defined tissue culture medium
- Explant tissue
- Methods for *in vitro* propagation
Sterile work

Pre-treatments for preparing explants

- Transfer plants to a greenhouse to reduce endemic contaminants
- Force outgrowth of axillary buds
- Remove surface contaminants by rinsing with sterilizing solutions
- Use detergents before sterilizing tissues.

Bacteria and fungi unless removed completely will overgrow the explants on the tissue culture medium.
Sterilants

There are some principal ways to kill surface contaminants

Oxidants
Active halogens
Heavy metal poisoning
Powerful chemicals such as conc. sulphuric acid may be used on seeds.
As far as possible, cut surfaces should be protected.

There is always a trade-off between killing surface contaminants and killing the explants
## Sterilants

<table>
<thead>
<tr>
<th>Sterilant</th>
<th>Concentration</th>
<th>Time</th>
<th>Action</th>
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<tbody>
<tr>
<td>NaOCl</td>
<td>10-20% v/v</td>
<td>10-20 mins</td>
<td>oxidant / Halogen</td>
</tr>
<tr>
<td>CaOCl</td>
<td>10-20% v/v</td>
<td>10-20 mins</td>
<td>oxidant / Halogen</td>
</tr>
<tr>
<td>H₂O₂</td>
<td>1% v/v</td>
<td>10 mins</td>
<td>oxidant</td>
</tr>
<tr>
<td>HgCl₂</td>
<td>0.1% w/v</td>
<td>10-30 mins</td>
<td>Heavy metal</td>
</tr>
<tr>
<td>AgNO₃</td>
<td>1% w/v</td>
<td>10-30 mins</td>
<td>Heavy metal</td>
</tr>
</tbody>
</table>

Antibiotics are rarely used since many are merely bacteriostatic. Massive overgrowth of cultures can result from residual bacteria and/or selection of resistances to antibiotics.

There are no antifungal compounds that are proven to be innocuous.
Sterile working conditions in a laminar flow hood
Growth chamber with controlled temperature and illumination
Terminology in plant tissue culture

**Callogenesis:** Callus Formation

**Embryogenesis:** Somatic Embryo Formation

**Organogenisis, Caulogenisis:** Shoot Formation

**Rhizogenesis:** Root Formation
Types of tissue cultures

Meristem culture (elongation of dormant meristems)

Embryogenesis (from somatic cells)

Organogensis (from callus, or directly on explants)

Adventitious Shoot Formation
Adventitious shoot formation is the *de-novo* development of shoots from cell clusters in the absence of pre-existing meristems.
Factors determining success with plants TC

Status of donor plant
- Species, genotype, age of tissue, explant size and type

Experimental conditions
- Temperature, light, day/night

Composition of culture medium

Important:
All factors are genotype-dependent and require optimization for each cultivar
Plant tissue culture media

Inorganic compounds (mineral nutrition)

Carbohydrates (typically sucrose)

Plant Growth Regulators (PGRs) (hormones)

Miscellaneous compounds

Plant tissues cultured \textit{in vitro} require a balanced supply of nutrients
Inorganic compounds

Macronutrients

- N, P, K, Mg, Ca, S

Micronutrients

- Mn, I, Cu, Co, B, Mo, Fe, Zn, (Ni, Al)
Murashige & Skoog universal tissue culture medium (MS medium)

<table>
<thead>
<tr>
<th>Macro Elements:</th>
<th>Micro Elements:</th>
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<tr>
<td><strong>CaCl$_2$</strong></td>
<td>*<em>CoCl$_2$<em>6H$_2$O</em></em></td>
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<tr>
<td>332.02 mg/l</td>
<td>0.025 mg/l</td>
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<tr>
<td><strong>KH$_2$PO$_4$</strong></td>
<td>*<em>CuSO$_4$<em>5H$_2$O</em></em></td>
</tr>
<tr>
<td>170.00</td>
<td>0.025</td>
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<tr>
<td><strong>KNO$_3$</strong></td>
<td><strong>FeNaEDTA</strong></td>
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<td><strong>H$_3$BO$_3$</strong></td>
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<tr>
<td>180.54</td>
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<tr>
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<td>1650.00</td>
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<table>
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<tbody>
<tr>
<td>myo-Inositol</td>
<td><strong>Glycine</strong></td>
</tr>
<tr>
<td>100.00 mg/l</td>
<td>2.00 mg/l</td>
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<tr>
<td>Nicotinic Acid</td>
<td><strong>Sucrose</strong></td>
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<tr>
<td>0.50</td>
<td>30000.00 mg/l</td>
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<tr>
<td>Pyridoxine HCl</td>
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</tr>
<tr>
<td>0.50</td>
<td><strong>Growth Regulators</strong></td>
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<tr>
<td>Thiamine HCl</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td></td>
</tr>
</tbody>
</table>

Sterilize by autoclaving (121°C, 15 min); pH 5.5 (after autoclaving)

Typical plant growth regulators and their effects on tissue cultures

- **Auxins**
  - callus
  - roots

- **Cytokinins**
  - shoots
  - embryoids

- **Gibberellic acids**
  - cell growth
  - elongation
Auxins

Synthesis in apex (from tryptophan)

IAA – free or in bound form

IAA- Sensitive to light (photooxidation) and IAA-oxidases

Synthetic auxins: 2,4-D, NAA

Indolyl Acetic Acid (IAA)

Naphthyl Acetic Acid (NAA)

2,4 Dichlorophenoxy Acetic Acid (2,4D)

Indolyl Butyric Acid (IBA)
Effects of auxins

Transport of Auxins is basipetal

(van der Weij, 1932)
Effects of auxins: Axillary bud formation

apical dominance

axillary buds develop
Effects of auxins: Adventitious root formation

Initiation of roots
Auxins

Plant growth and physiological functions
  Phototropism
  Apical dominance
  Cell division
  Differentiation
  Initiation of embryos, organs (esp. roots)

Synthetic auxins are often more effective than the natural auxins.
Cytokinins

Purine-type cytokinins
Synthesis from adenine in root tips, embryos, young fruits, leaves in all plants
Natural: Zeatin
Synthetic: BAP

Non purine-type cytokinins

Kinetin

Benzylaminopurine (BAP)

Thidiazuron (TDZ)
Cytokinins
Main effects in tissue culture systems

Adventitious shoot formation (at high conc.)
Inhibition of root formation
Cell division
Callus formation and growth
Stimulation and outgrowth of axillary buds
Inhibition of leaf senescence
Auxins and cytokinins act synergistically

Growth regulators

IAA: 3.0 mg/l 3.0 mg/l 0.03 mg/l 0.0 mg/l
Kinetin: 0.2 mg/l 0.02 mg/l 1.0 mg/l 0.2 mg/l

Nutrient agar
Effect of cytokinins and auxins on senescence

Incubation in

<table>
<thead>
<tr>
<th>Kinetin [µM]</th>
<th>0</th>
<th>10</th>
<th>0</th>
<th>10</th>
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</thead>
<tbody>
<tr>
<td>Auxin [µM]</td>
<td>0</td>
<td>0</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

7 days later
Effects of auxins and cytokinins

**High Auxin**
- Root formation on cuttings
- Callus initiation in monocots.
- First stage of embryogenesis
- Adventitious root formation from callus

**Low Auxin**
- Callus initiation in dicots.
- Adventitious shoot formation
- Axillary shoot proliferation in shoot cultures

**High Cytokinin**
- ... (Text not fully visible)

**Low Cytokinin**
Gibberellic acid (GA)

Synthesized from mevalonate shoot and root apices, embryos, cotyledons, fruit, tubers

Only some forms are biologically active: GA₃

Dramatic effects on cell elongation

Promotes cell division in combination with IAA

Effects on seed germination (breaking seed dormancy)

Improves fruit set, fruit growth, fruit maturation and fruit ripening

Promotes flowering
Effects of Giberellic Acid (GA₃)
GA inhibitors ('Antigibberellins')

Influence of anti-gibberellins on plant height of *Chrysanthemum* spec.
Liquid media and support systems

Liquid medium

- Protoplast cultures
- Suspension cultures
- Homogenous distribution of nutrients

- Oxygen deprivation
- Hyperhydration

Laboratory – large scale cultures bioreactor
Liquid media and support systems

Semi-solid medium

Widely used for
- Protoplast cultures
- Cell, tissue and organ cultures

Nutrient gradient

Gelling agents
- Agar
- Gellan Gum
Gelling agents

Agar

Unbranched polysaccharide (red algae; Gelidium)
Melts at approx. 100°C, solidifies at approx. 45°C
Concentrations 0.5 – 1.0%
Gels are not digested by plant enzymes
Does not strongly react with media constituents
Contains impurities
Gelling agents

Gellan Gum (Gelrite™, Phytagel™, Kelcogel™)

Exopolysaccharide (*Pseudomonas elodea*)

$\text{Ca}^{2+}, \text{Mg}^{2+}$

Clear gel

May cause hyperhydricity
Summary

Plant Biotechnology

Initiation of plant cell cultures

Culturing of plant cells

Growth conditions

Plant tissue culture media

Plant growth regulators
Environmentally Friendly Production

Nutrition
- vitamins

New Materials
- PHBV

Biomass
- fuel
- starch
- paper

Health
- vaccines
- essential oils
- prenylated flavonoids

Flavors, Fragrances, Dyes