# *Agrobacterium*-mediated Transformation of Sunflower cells – Techniques & Experimental Setup

**Katja Geipel[1], Joachim Püschel[2], Jutta Ludwig-Müller[2], Thomas Bley[1]**

[1]Institute of Food Technology and Bioprocess Engineering, Technische Universität Dresden, Bergstraße 120, 01069 Dresden, Germany

[2]Institute of Botany, Chair of Plant Physiology, Technische Universität Dresden, Zellescher Weg 20b, 01217 Dresden, Germany

## Introduction:

- **Secondary plant metabolites:** variety enormous
  - ► Application e.g. in pharmacy & cosmetics
- Project `**Plant Cells in White Biotechnology**´: Investigation of technology platforms for industrial transfer of plant biotechnology
- **Modern biotechnology**: production independent from environmental factors
- Combination of **bioprocess** & **genetic engineering**

Fig. 1: Sunflower - *Helianthus* sp.

## Aim:

- **Plant cell cultures** (suspension) with enhanced production of biological active substances
- **Application** in biotechnological production processes
- **Transfer** of process from lab into industry scale
- Modell organism: **Sunflower** (Fig. 1)
  - ► α-tocopherol (vitamin E, E307) [1]

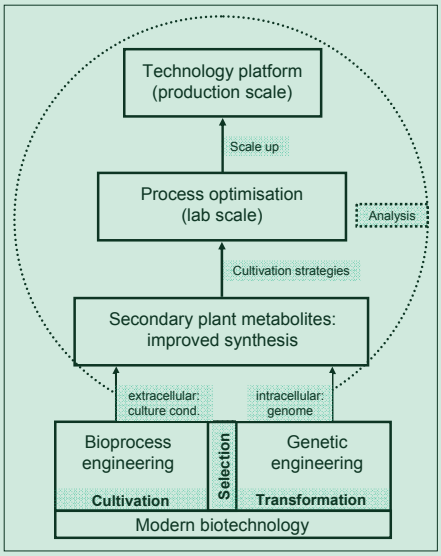




Fig. 2: *A. tumefaciens* adhering to plant cells [3]

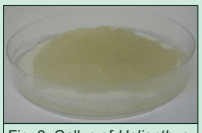


Fig. 3: Callus of *Helianthus annuus*, appr. 2 weeks old

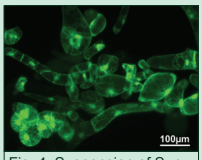



Fig. 4: Suspension of Sunflower, stained with fluorescence diacetate FDA (fluor. microscopy, vital cells are green)

## Experimental design:

### 1. Transformation

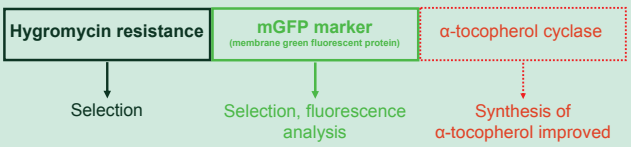
- Favoured plasmid: pJP-At-VTE1 (Fig. 5)
  - ► α-tocopherol cyclase & Hygromycin resistance
- *Agrobacterium tumefaciens* GV3103::pMP90 (Fig. 2) with control plasmid pCambia1302
  - ► Pre-culture in YEB with 10 µg/mL Gentamycin & 50 µg/mL Canamycin
- *Helianthus annuus* (Fig. 3, 4)
  - ► Suspension in **LS** with 0,2 µg/mL **2,4-D**ichlorphenoxyacetic acid & 0,1 mMol/L Acetosyringon
- Co-cultivation with $OD_{bact.}$ = 0.13; 0.50 & 1.04: dark, 110 rpm & 26°C [2] for 3, 24 & 48h
- Killing of bacteria after transformation: 250 µg/mL Cefotaxime

### 2. Selection

- Investigation of required Hygromycin conc.: 25-250 µg/mL
  - ► Selection of transgenic plant cells
- Analysis with flow cytometry & fluorescence microscopy

### 3. Cultivation, Analysis

- Cultivation in shaking flask & stirred tank reactor
  - ► Optimisation of cultivation conditions (e.g. temperature, media, day/night rhythm), process development, scale up
- GC/MS-analysis of α-tocopherol




see also: Püschel *et al.* Alpha-Tocopherol Production in Sunflower Callus Cells and Hairy Roots: A Genetic Approach, Poster at PTT II 2011.

Fig. 5: Schematic overview of plasmid pCambia1302 (dotted: α-tocopherol cyclase to be inserted in plasmid pJP-At-VTE1 which not includes mGFP marker)

## Results:

- Setup for further transformation experiments with plant cell suspensions:

  round-bottomed flask, glas funnel & cotton filter, sterile metal spoon & tweezers (Fig. 6)
  - ► Easy & sterile transfer of transgenic plant cells into bacteria-free medium after transformation
  - ► Separation of plant cells from bacteria successful
- Genome transfer from *A. tumefaciens* GV3103::pMP90 to *H. annuus* not successful
- Determination of Hygromycin concentration: 25 µg/mL
- Cefotaxime concentration sufficient for killing of bacteria in suspensions after transformation experiment

Fig. 6: Device for separation of plant cells from bacteria

## Future prospects:

- Possibillities for successful transformation: enzymatic digest of cell wall, variation of co-cultivation time, $OD_{bact.}$ etc. in progress
- Process development & optimisation of cultivation conditions
- GC/MS-analysis: Calibration successful, optimisation of derivatisation & extraction from plant samples ongoing

## References:

[1] A. Pavlov, S. Werner, M. Ilieva, T. Bley: Characteristics of *Helianthus annuus* Plant Cell Culture as a Producer of Immunologically Active Exopolysaccharides, Eng. Life Sci. 5. No. 3, 2005.

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TECHNISCHE UNIVERSITÄT DRESDEN
Institut für Lebensmittel- und Bioverfahrenstechnik

**Contact:**
Dipl.-Ing. Katja Geipel
Phone: +49 351 463 39042
Katja.Geipel@TU-Dresden.de

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