

Perspectives of using cell, hairy root and sprout cultures in food technology



Smetanska

Health-promoting secondary metabolites

glucosinolates

phenolic acids

anthocyanins

steviosides

Plant meta-bolite profile

System
-plants
- by-products,
- sprouts,
- in vitro (hairy roots, shoot, cell cultures)

Secondary metabolites

Synthesis
• nutrients
• phyto-hormons
• precursors
• elicitors

Extraction
• solvents
•exudation
• membrane permeabilisation

Properties
• antioxidative potential,
• stability (pH, temperature),

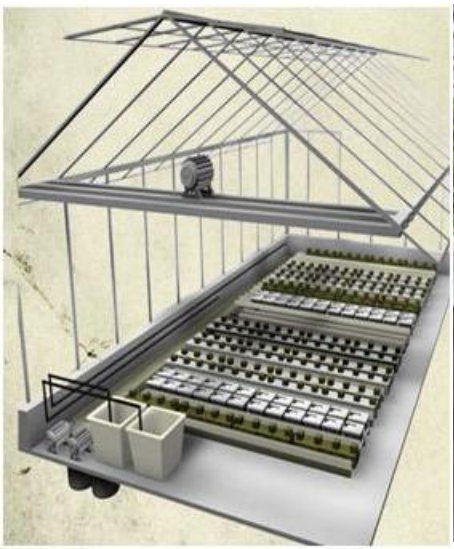
Phytomanufacturing chain

Smetanska, Adv. Biochem. Engineering, Springer (2008)

Cutivation systems

in vivo

Hydro- und Aeroponic

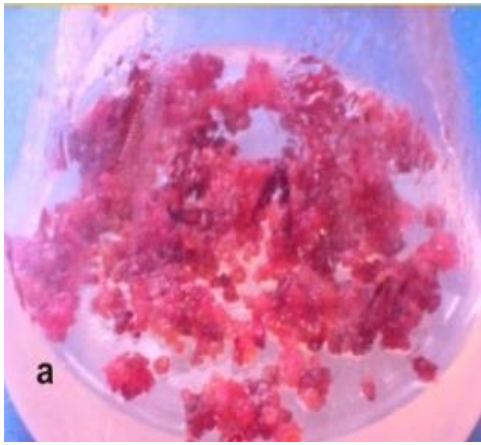


Food

in-vitro

Sprout culture

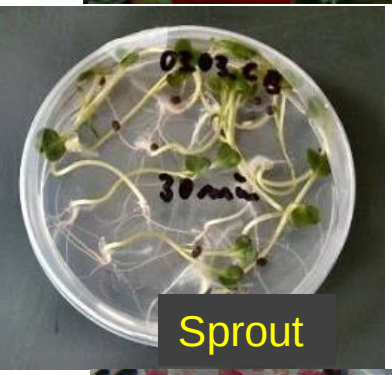
Cell culture

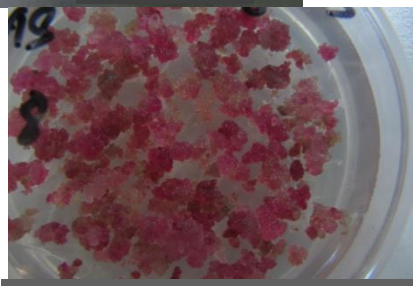


Hairy roots

Cultivation system: Cell cultures

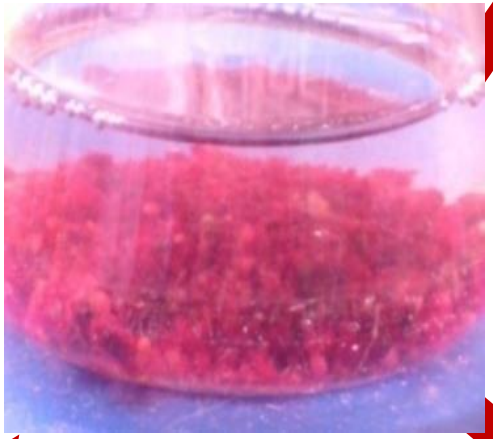

Plant


Sprout


Primary callus


Callus culture

1. Model system


Suspension

2. Propagation


Regeneration


Modifikation

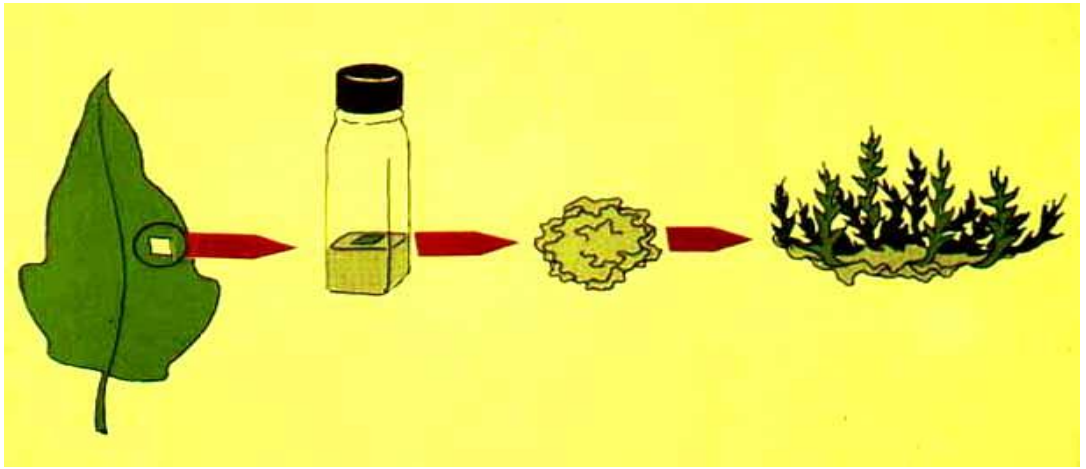
3. Metabolite production


Bioreactor

Cell cultures: Micropropagation

- **production of large numbers of plants from small pieces of the stock plant in relatively short periods of time**
- **similarly to any own-rooted vegetative propagated plant**

Orchids:
1 plant – 500 000 plantlets
vegetative propagation – 10 plantlets
1 m ^{2} – 100 000 plants

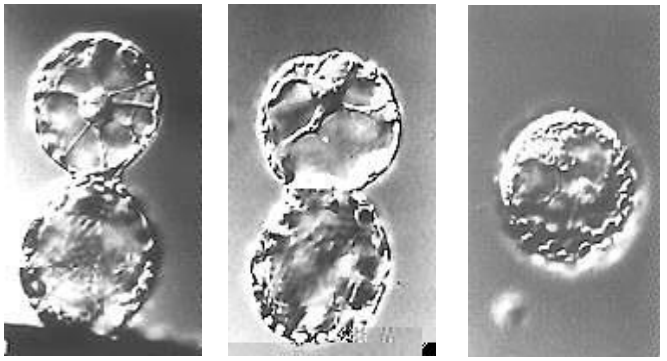


Differentiation from different plant parts aggie-horticulture.tamu.edu/.../pltissue.html

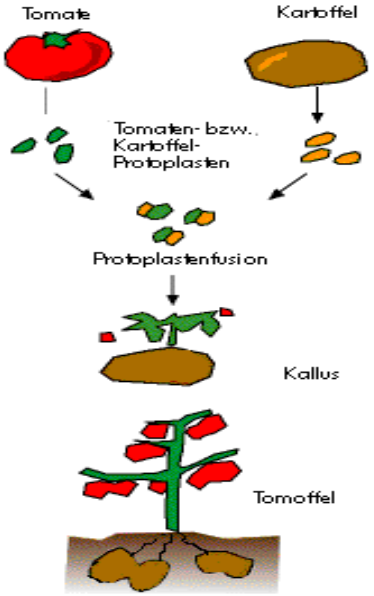
Cell cultures

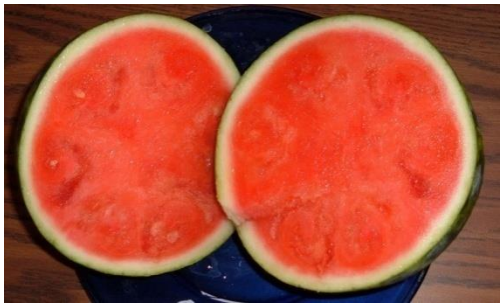
ENHANCED:

diversity (color)
stress or pest resistance
productivity
pathogen-free plants



Fusion of Protoplasts (KOOP, SCHWEIGER, 1985)





seedless watermelon (3 x)

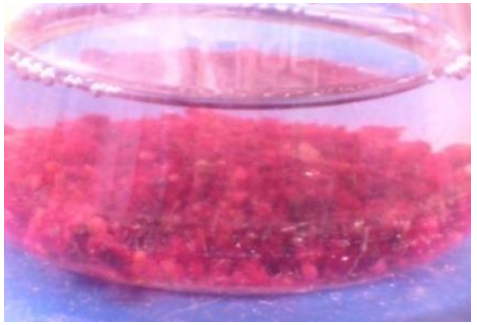
triticale (wheat x ray)

tomapo (tomato x potato)

http://lbs.hh.schule.de/biotech/pflanzen/

Cultivation system: Cell cultures

AROMA	
Coffea arabica	Coffee aroma
Malus sylvestris	Apple
Mentha Spezies	Mint
Theobroma cacao	Cacao
Vanilla planifolia	Vanilla
SWEETENER	
Stevia rebaudiana	Stevioside - Sugar
FARBSTOFFE	
Citrus limon	Flavonoide
Daucus carota	Red - Carotenoids



Product	Plant	Yield (% TG)		Cells/ Plant
		Cells	Plant	
Anthocyane	*Vitis vinifera*	16	10	1.6
	Euphorbia mille	4	0.3	13.3
Berberine	*Coptis japonica*	13	4	3.3
	Talictrum nomur	10	0.01	1000
Shikonin	*Lithospermum erythrorhizon*	14	1.5	9.3

Cultivation system: Cell cultures

Plant-derived pharmaceuticals of importance

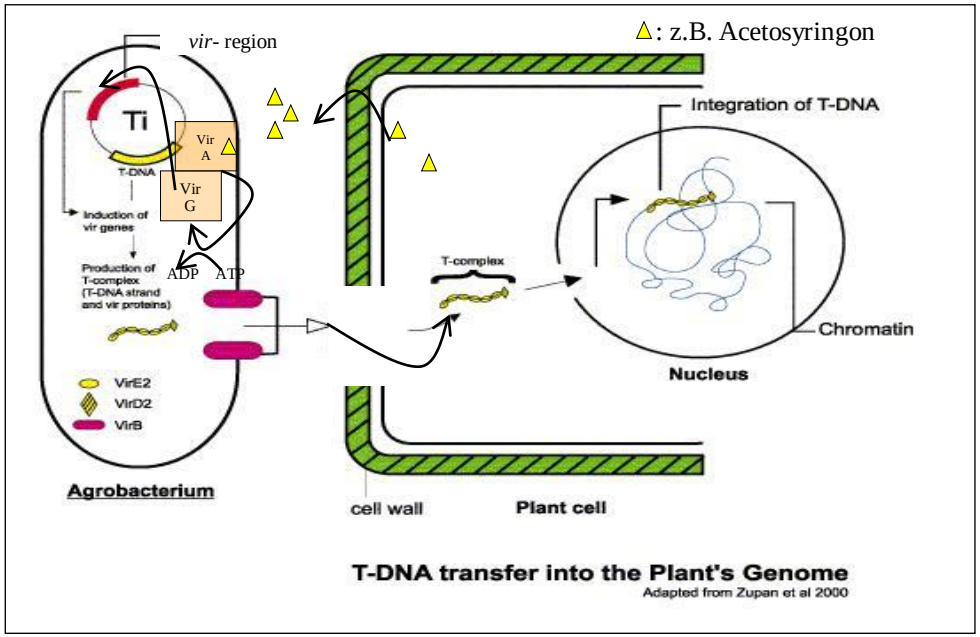
Product	Use	Plant species	Cost US$ kg^{-1}
Ajmalicine	antihypertensive	Catharanthus roseus	37 000
Ajmaline	antimalarial	Rauvolfia serpentine	75 000
Camptothecin	antitumour	Camptotheca acuminata	432 000
Codeine	sedative	Papaver somniferum	17 000
Colchicine	antitumour	Colchium autumnale	35 000
Ellipticine	antitumour	Orchrosia elliptica	240 000
Morphine	sedative	Papaver somniferum	340 000
Shikonin	antibacterial	Lithospermum erythrorhizon	4500
Taxol	anticancer	Taxus brevifolia	600 000
Vinblastine	antileukemic	Catharanthus roseus	1 000 000
Vincristine	antileukemic	Catharanthus roseus	2 000 000

Cape periwinkle
Catharanthus roseus

opium poppy

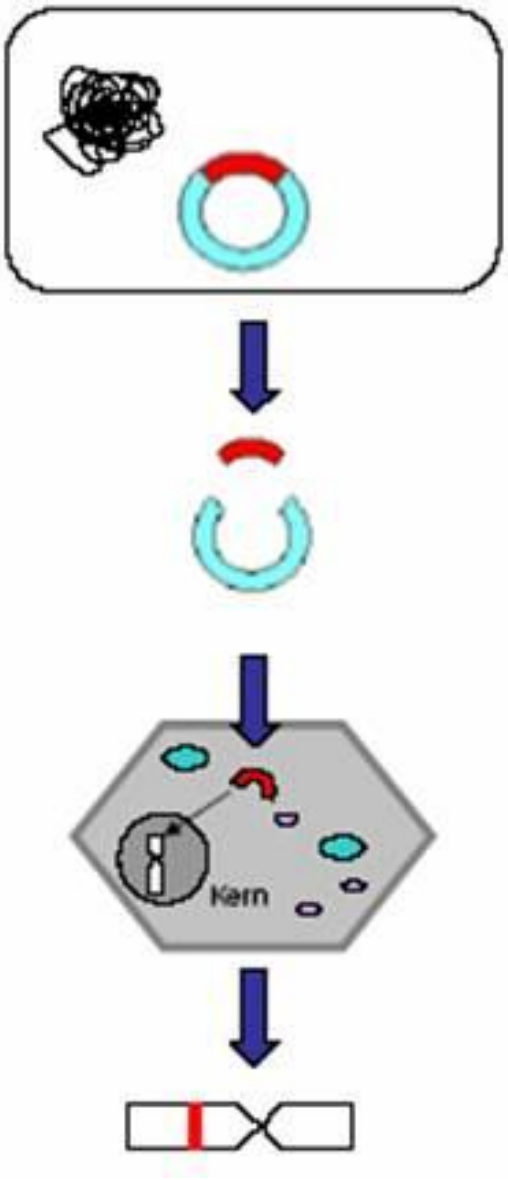
Pacific yew

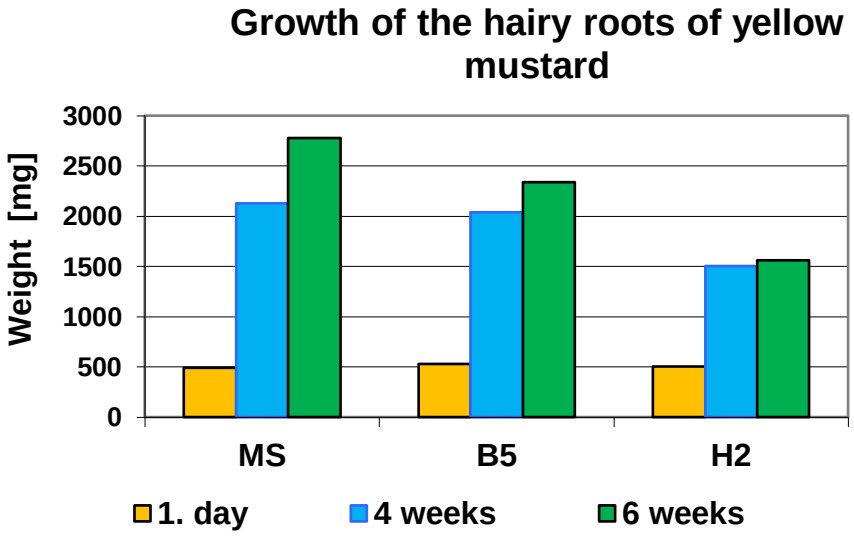
Cultivation system: Hairy roots



Cultivation systems: Hairy roots

Agrobacterium rhizogenes: LBA 9402 and A4





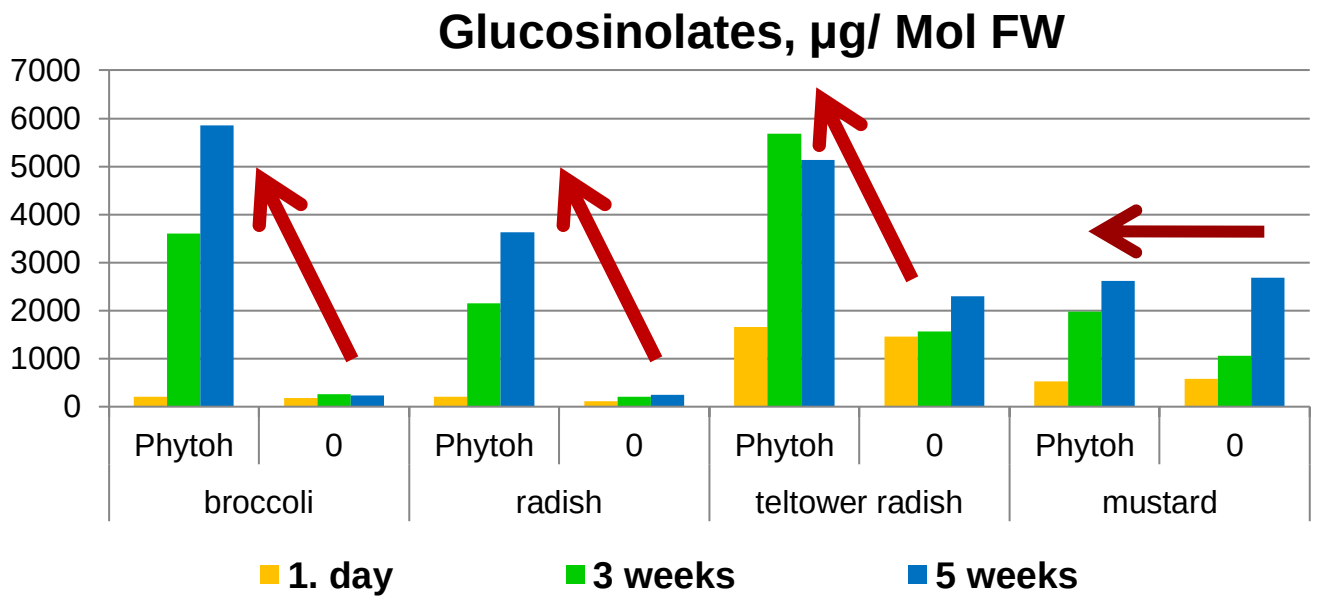
BMBF- Kastell, Smetanska, 2009

Cultivation systems: Hairy roots

Phytohormons

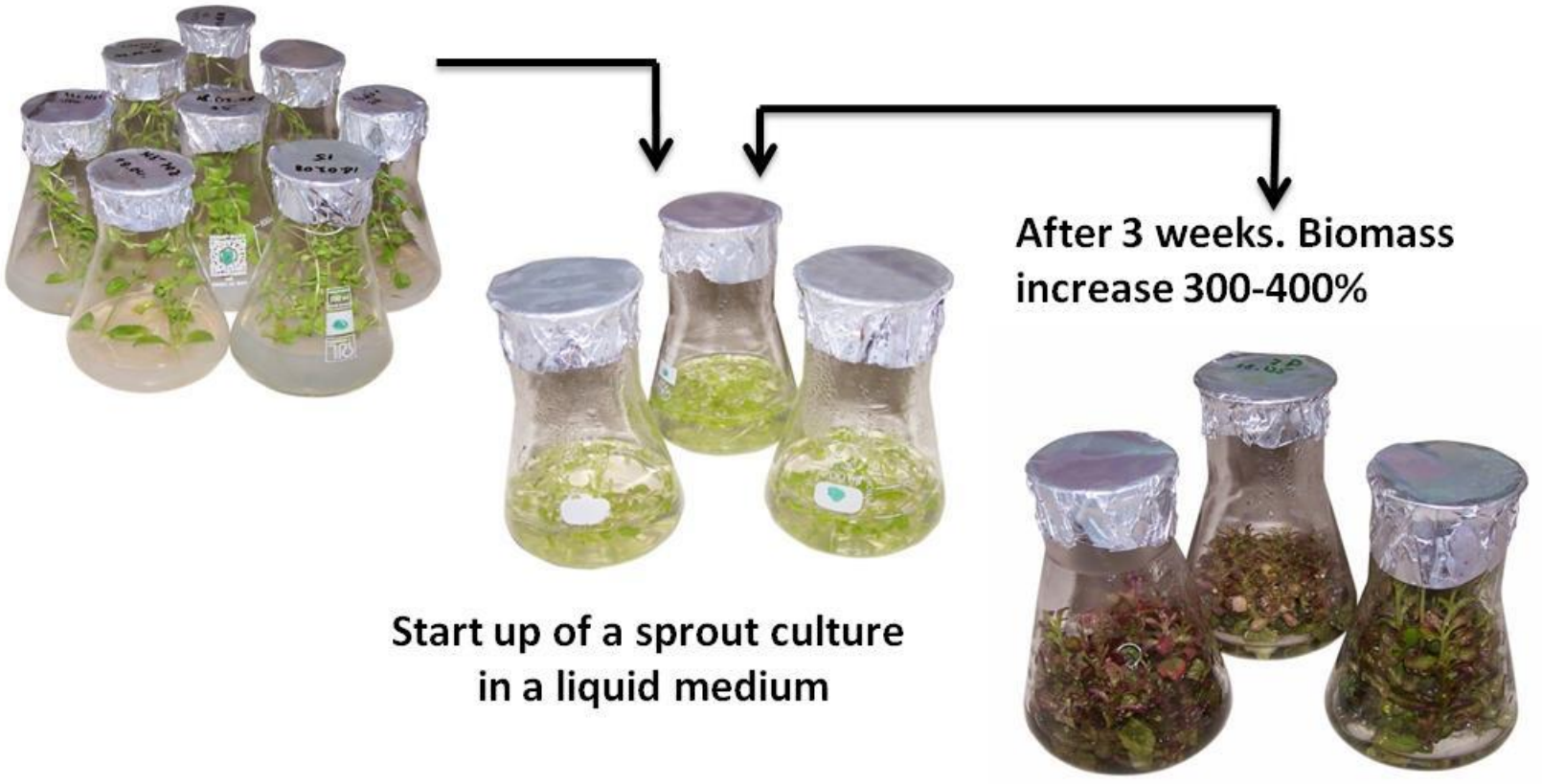
Mix
- **NAA**
- **Kinetin**
- **2,4-D**

Change in morphology / content of glucosinolates



Cultivation systems: Sprouts

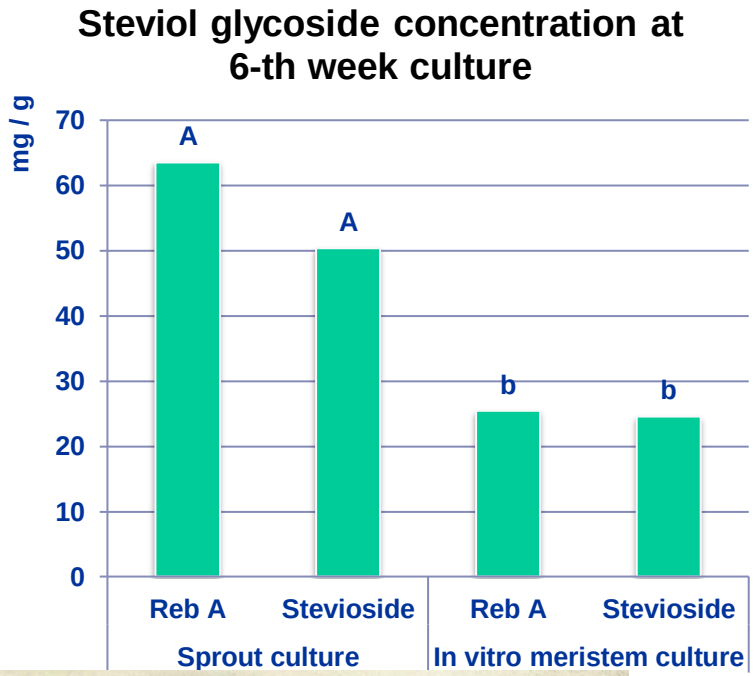
- production of large numbers of plants from small pieces of the stock plant in short periods of time
- similarly to own-rooted plant



Schewchenko, Smetanska, 2008

Cultivation system: Sprout culture

1. week 2. week 4 . week



BPM; BMBF- Schevchenko, Wendt, Smetanska, 2008

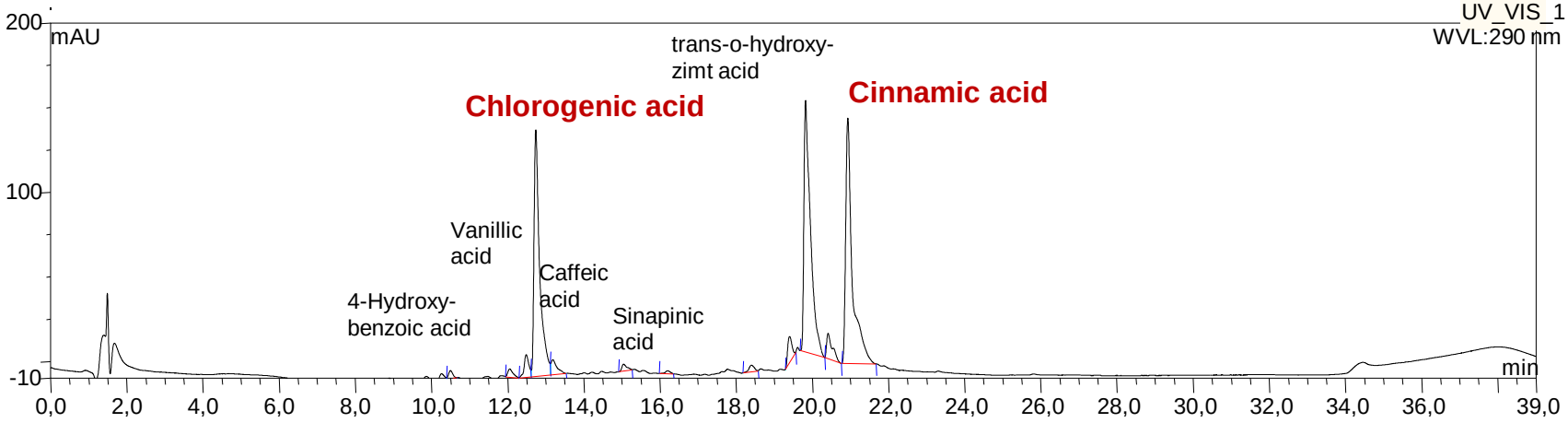
Cultivation systems

Plants | Shoot cultures | Hairy roots | Cell cultures

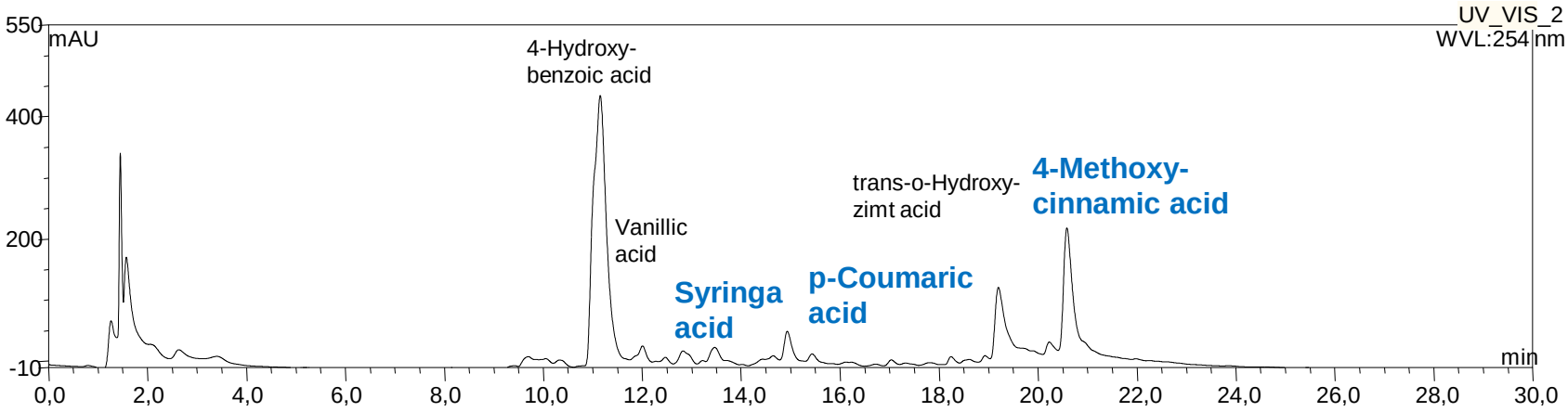
Advantages and disadvantages	Plants	By-products	Shoot cultures	Hairy roots	Cell cultures
Independence of season	-	-	+	+	+
Controlling	-	-	+	+	+
Growth intensity	+	+	+	+/-	+/-
Maintaining costs	+	+	-	-	-
Growth equality	+	+	+	+	+/-
Costs for extraction	-	-	+	+	+

Cultivation systems: Cell cultures

+ *de novo* synthesis

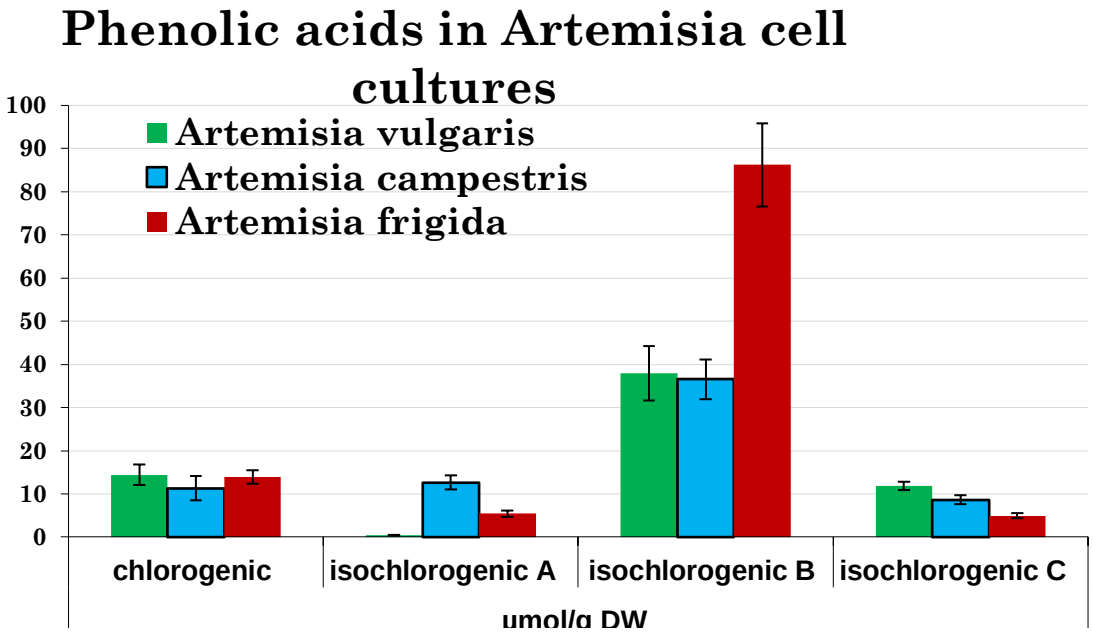


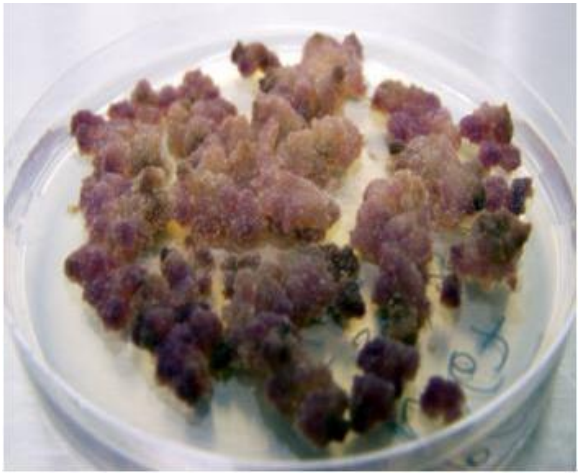
HPLC chromatogram of the cell culture of *Echinacea angustifolia*

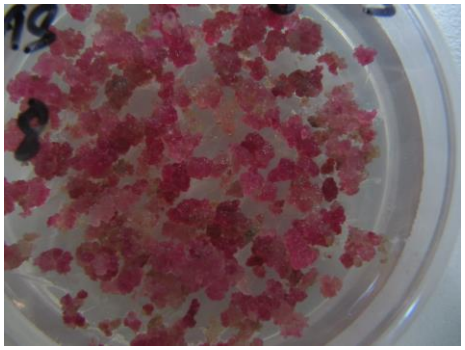


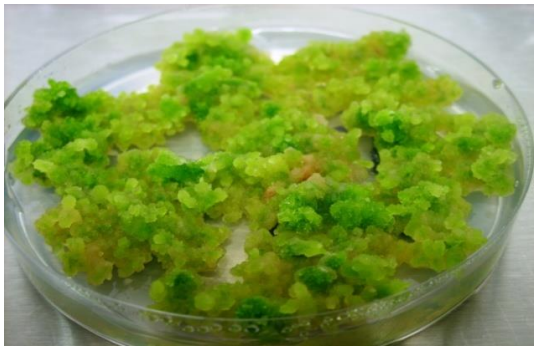
HPLC chromatogram of the parent plant *Echinacea angustifolia*

Cultivation systems: Cell cultures









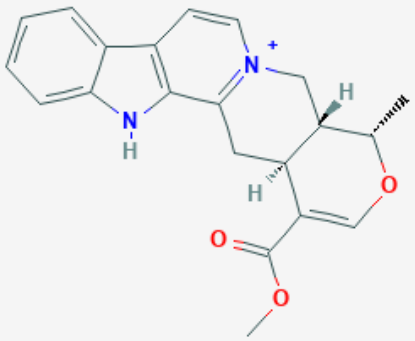
Cai. Riedel, Smetanska, 2009

Synthesis: nutrients

Media for plant cell cultures (mg l^{-1})

Components	MS	HV	MSR + BA/NAA	MSR + 2,4D
Makroelemente				
KNO_3, NH_4NO_3, KH_2PO_4	XXX	XXX	XXX	XXX
$CaCl_2 \times 2H_2O$, $MgSO_4 \times 7H_2O$	XXX	XXX	XXX	XXX
Mikroelemente				
$MnSO_4 \times H_2O$, $ZnSO_4 \times 7H_2O$	XXX	XXX	XXX	XXX
H_3BO_3, KJ, $CoCl_2 \times 6H_2O$	XXX	XXX	XXX	XXX
$NaMoO_4 \times 2H_2O$, $CuSO_4 \times 2H_2O$	XXX	XXX	XXX	XXX
Vitamine				
Myo – Inositol, Nicotinsäure	XXX	XXX	XXX	XXX
Glycin	XXX		XXX	XXX
Pyridoixin x HCl, Thiamin x HCl	XXX	XXX	XXX	XXX
Phytohormone				
Dichlorphenoxyessigsäure (2,4D)	XXX	XXX		XXX
Naphtylessigsäure (NAA)			XXX	
Benzyladenin (BA)			XXX	
Kohlenstoffquelle - Saccharose	XXX	XXX	XXX	XXX

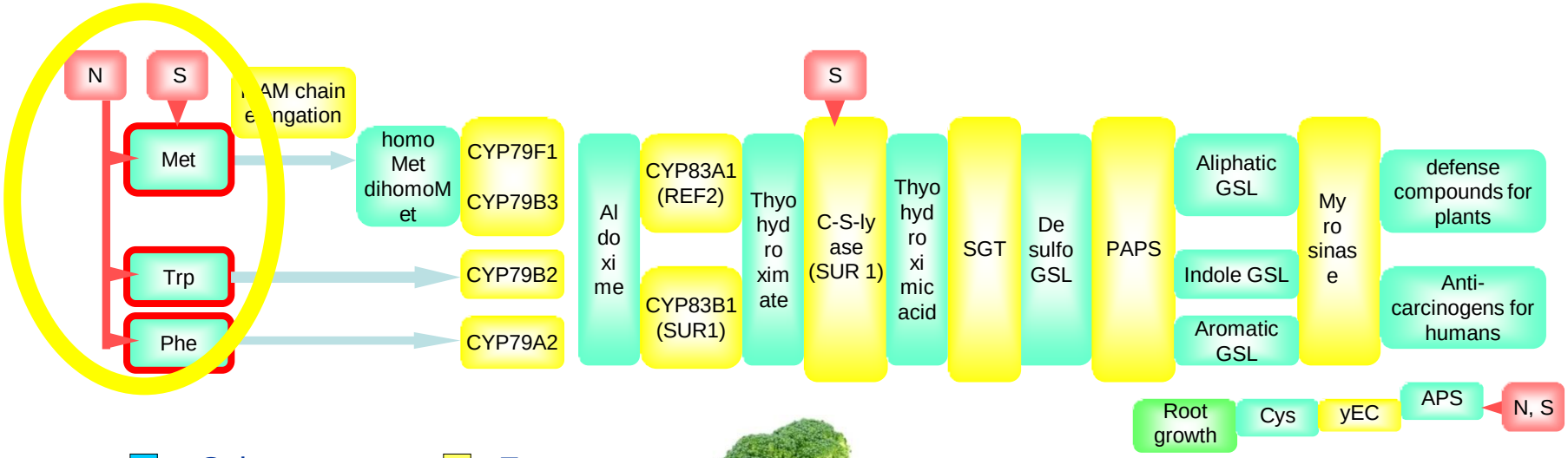
Synthesis: nutrients



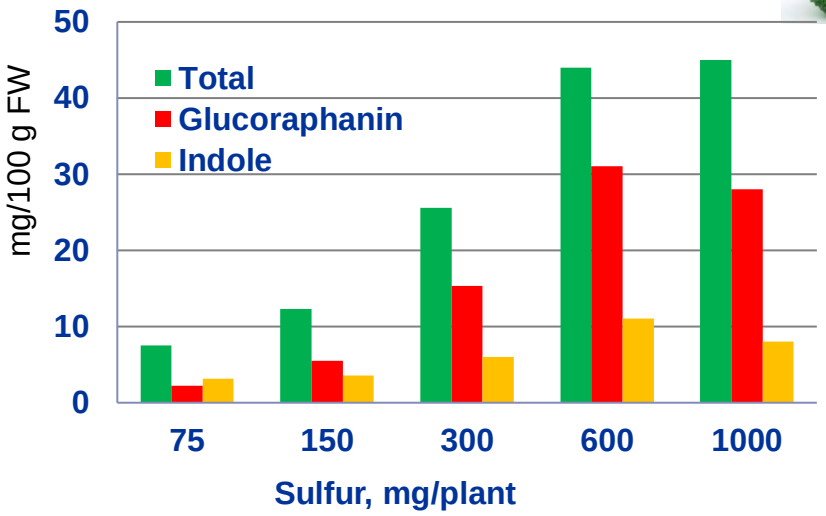
Effects of different media on growth and serpentine production in cell suspension cultures of *Catharanthus roseus* (Zenk, 2004)

Basal medium	Cell yield g DW l^{-1}	Serpentine mg l^{-1}	Serpentine, % DW
Blaydes	7.6	4.4	0.06
Gamborg - B5; + 2,4-D (l mg l^{-1})	4.6	0.5	0.01
Gamborg + 2,4 D (2 mg l^{-1})	5.2	0	0
Gamborg + NAA (1.86 mg l^{-1})	7.6	1.2	0.02
Gamborg	5.1	0	0
Heller + IAA (0.17mg l^{-1}); BA (1.1 mg l^{-1})	5.4	6.6	0.12
Linsmaier and Skoog	9.3	0	0
Murashige and Skoog (N, K)	8.9	10.4	0.12
Nitsch and Nitsch	2.3	2.0	0.09
Velicky and Martin	5.0	0	0
White	0.8	0	0

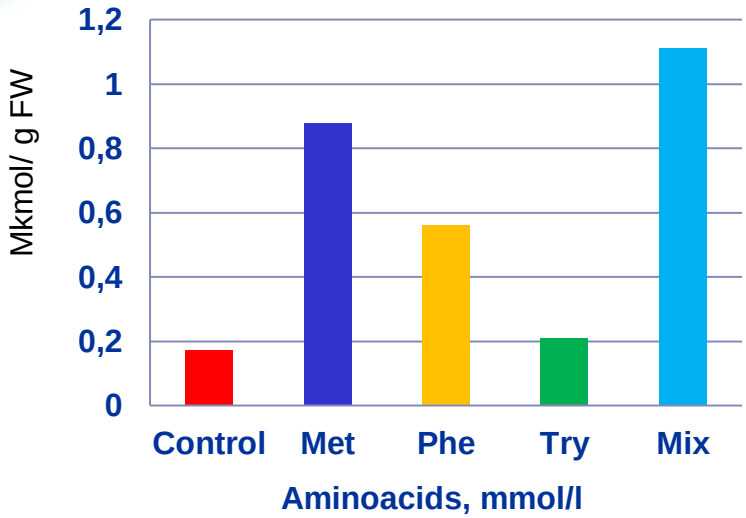
Synthesis: nutrients, transformation



Glucosinolates and sulfer



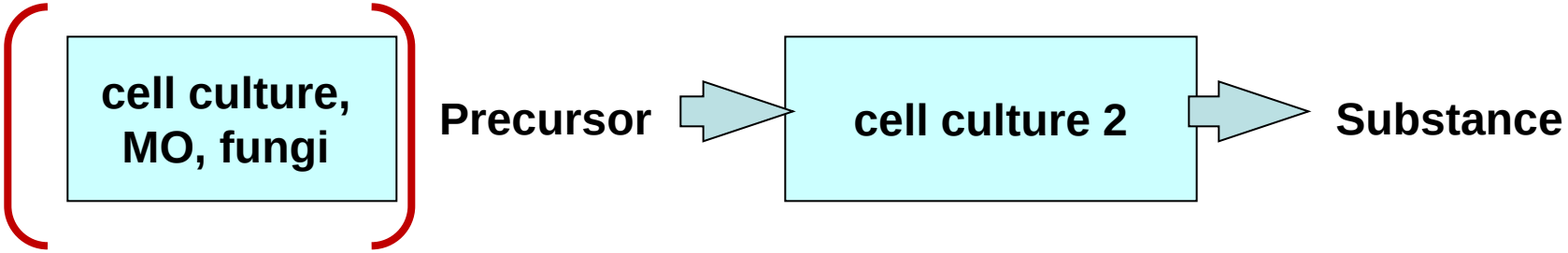
Glucosinolates and aminoacids



Leibnitz Institute of Vegetable and Ornamental crops – WG Schreiner; BMBF- Kastell, Smetanska

Biotransformation / co-cultivation

Problem: Abscence of precursors



1. **chemical**

PCC Vitis vinifera + L-Phenylalanin (1,5-10 µM) = Anthocyan (2,2-times)

2. **biological - plants:**

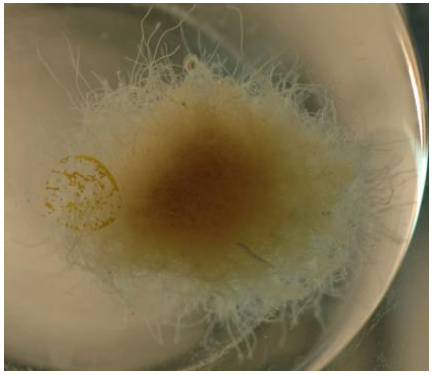
a. **from plants / cell cultures of the same specie**

b. **from plants / cell cultures of the other specie**

3. Biological – extract of microorganisms / bacteria / fungi - extracellular production of precursors

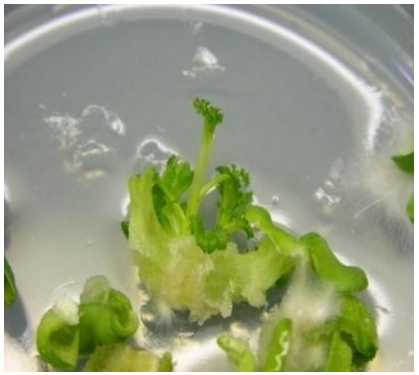
- **transformation with *Agrobacterium* – hairy roots**
- ***PCC Gossirium arboreum + Verticullum = gossypol***
- ***PCC Origanum vulgare + Pseudomonas = new synthesis of phenolics***

Synthesis: phytohormones



Hairy roots - *Brassica rapa*

1. **Auxins – stretching growth formation of adventives roots**
2. **Gibberellins – growth germination**
3. **Cytokinins – cell division, formation of side buds**
4. **Ethylene - fruit maturation, fruit and leaf fall**
5. **Abscise acid - suppress growth, antagonist to 1-3**
6. **Jasmone acid – induction of defence-genes, allelopatic interactions**
7. **Brassinosteroids – increase resistance to stress**



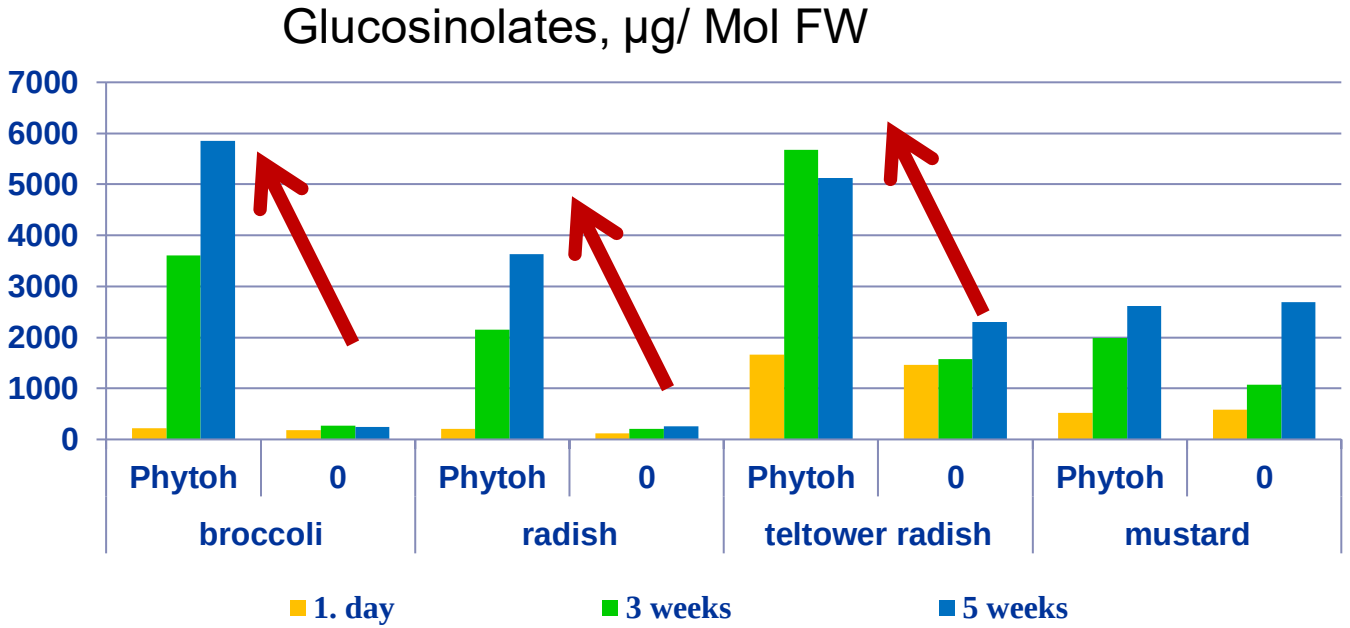
Cell cultres- *Brassica rapa*

Synthesis: phytohormones

Brassica oleracea

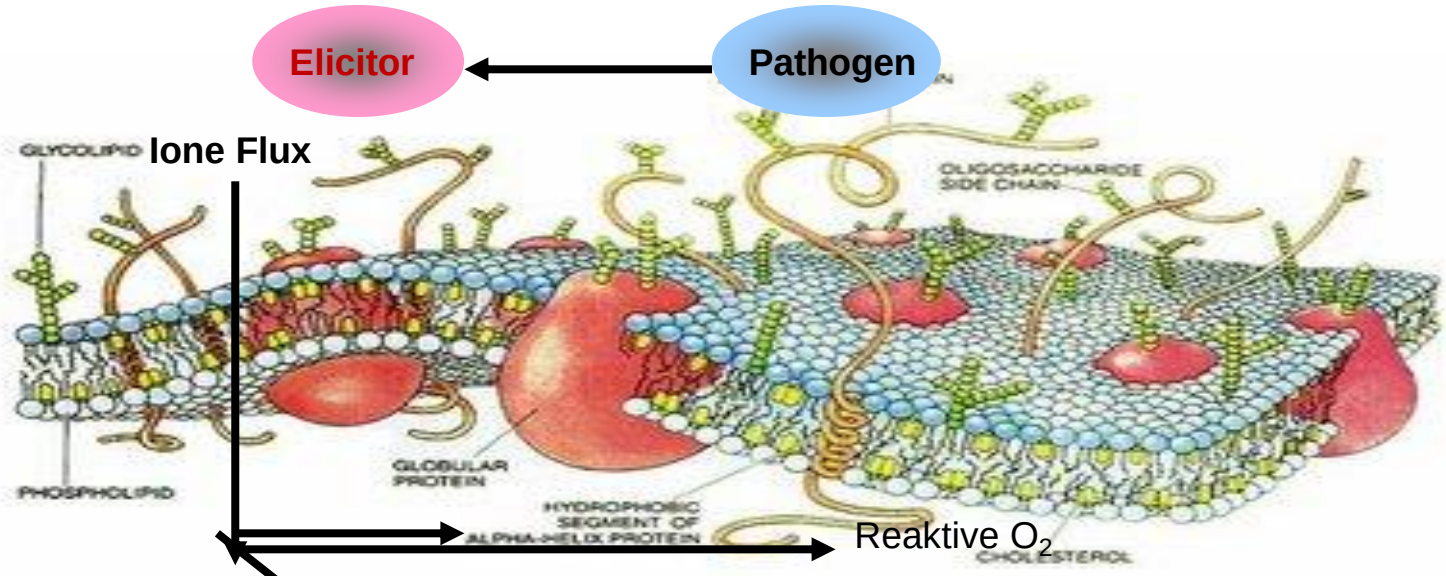
without 2,4-D

with 2,4-D



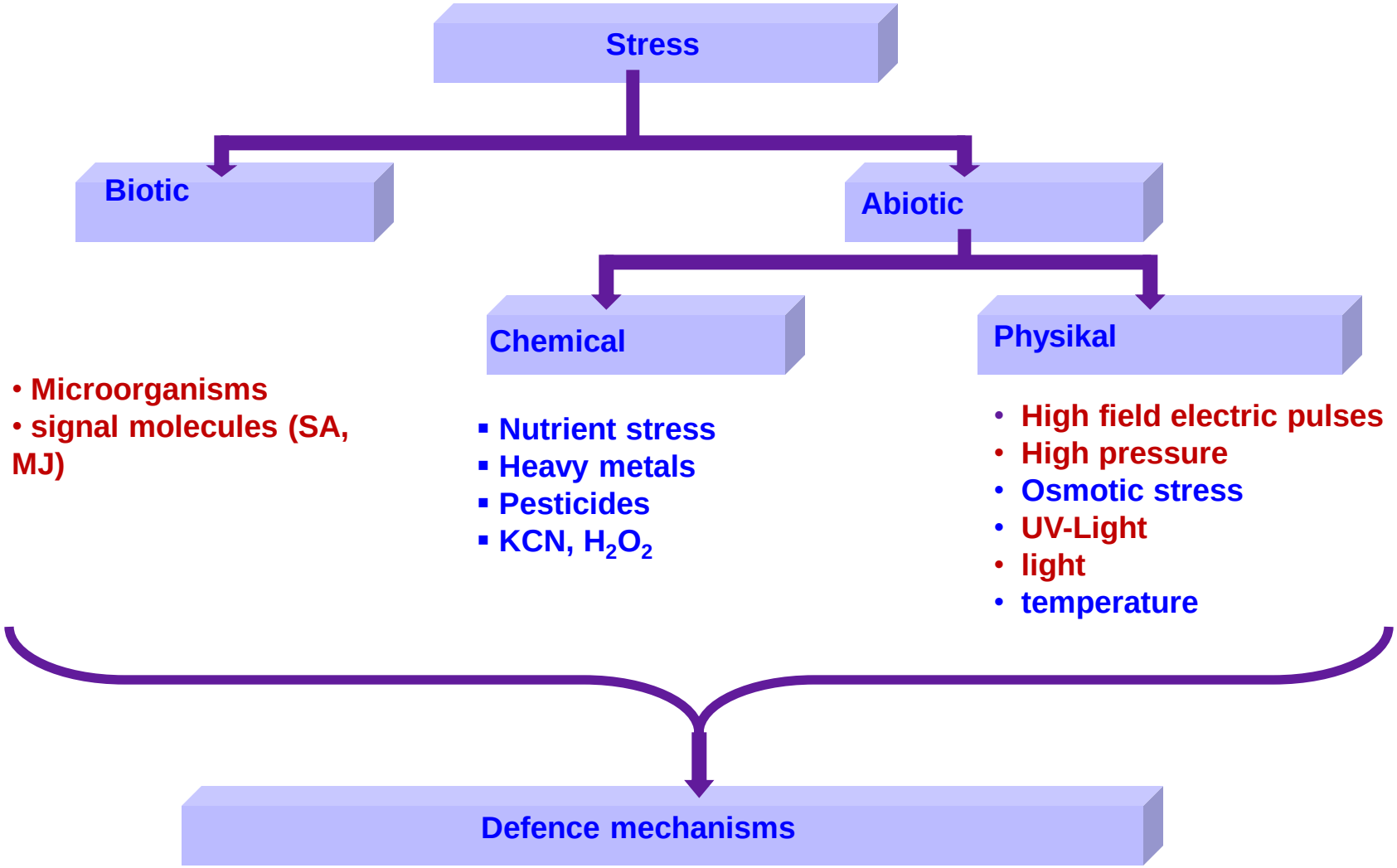
Synthesis: stress factors

Answer on pathogen attack:
Recognition of pathogen,
Induktion of defence mehanisms
Synthesis of secondary metabolites

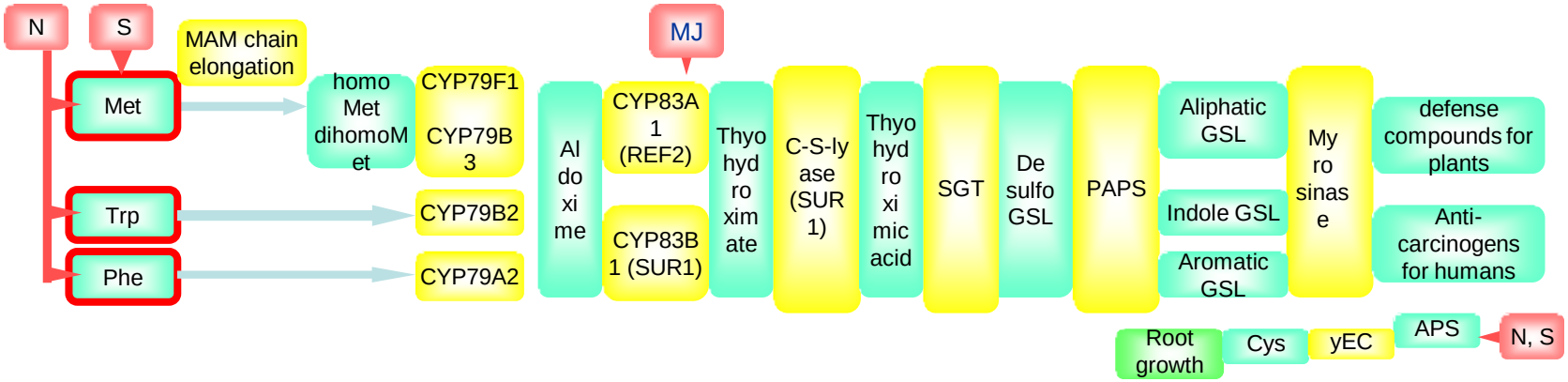


Knorr, 1995

Synthesis: Elicitors



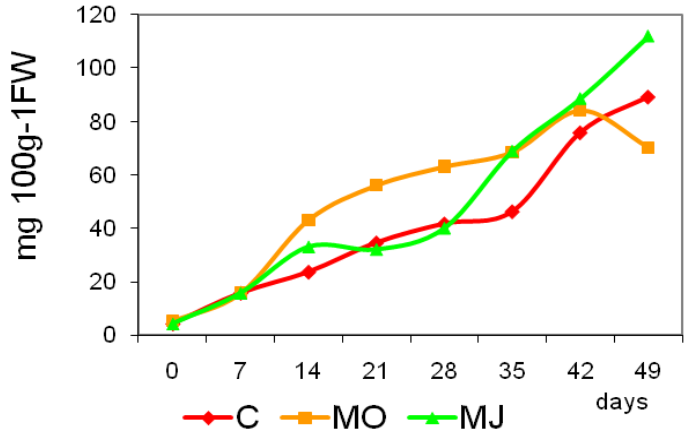
Synthesis: stress factors



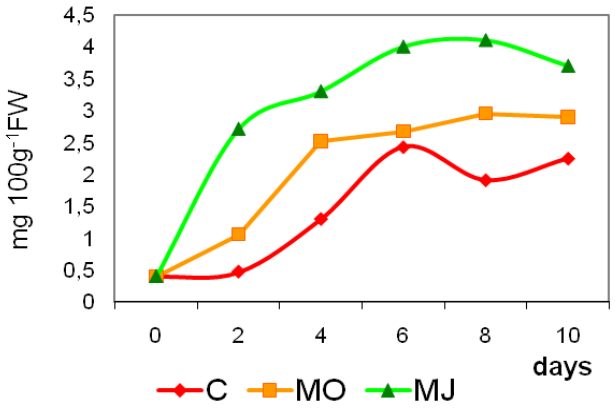
- substances, - enzymes (CYP- cytochrome 450 type), -elicitors

Halkier, 2006; Kliebestein et al., 2005

Glucosinolates in plants



in hairy roots



C - control, MO - microorganisms, MJ - methyl jasmonate (Smetanska *et. al*., 2013)

Extraction: Immobilisation

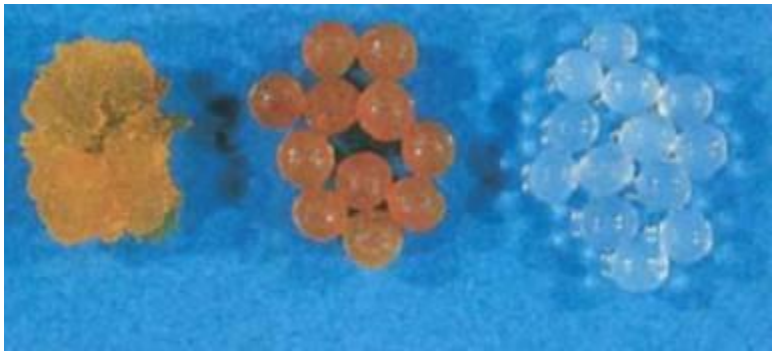
Immobilised cells – limited in mobility

a. **Naturally immobilised - 100-500 µm (some cells) until some mm (thousands of cells)**

b. **Artificially immobilised - in particles from alginate, pectin, agar, gelatine**

- Influence potential for synthesis (cell-cell contact, predifferenciation)

- Continuous cultivation



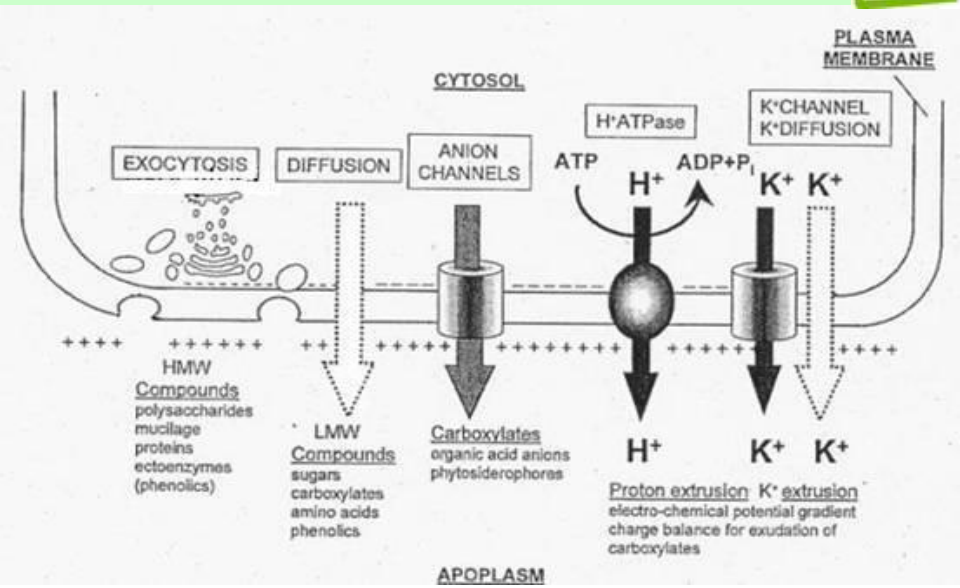
Extraction: exudates

Rhizosecretion

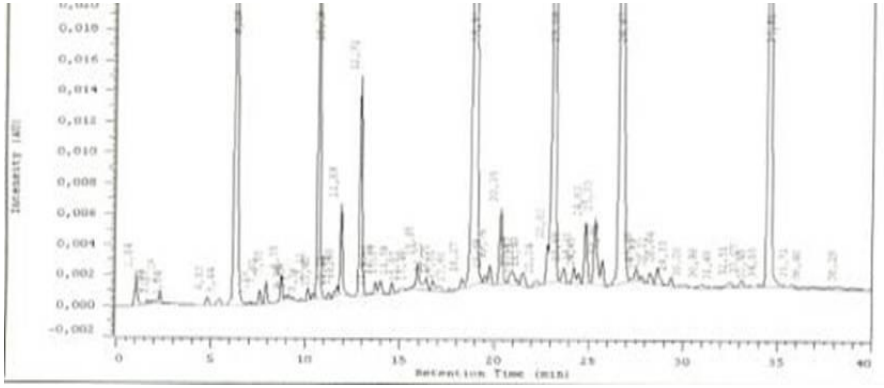
- simplicity of extraction
- decrease costs for purification
- continuity
- constant profile of metabolites

Ways of rhizosecretion enhancing

- direct - increase root surface area
- indirect - increase glucosinolate concentration in roots

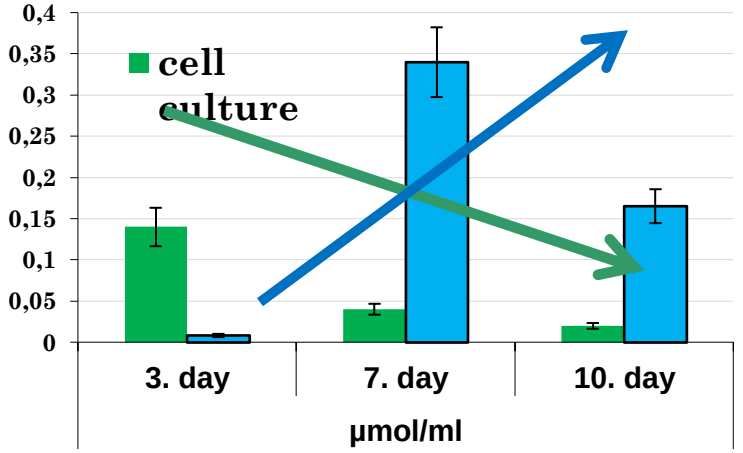


Glucosinolates in exudates of turnip



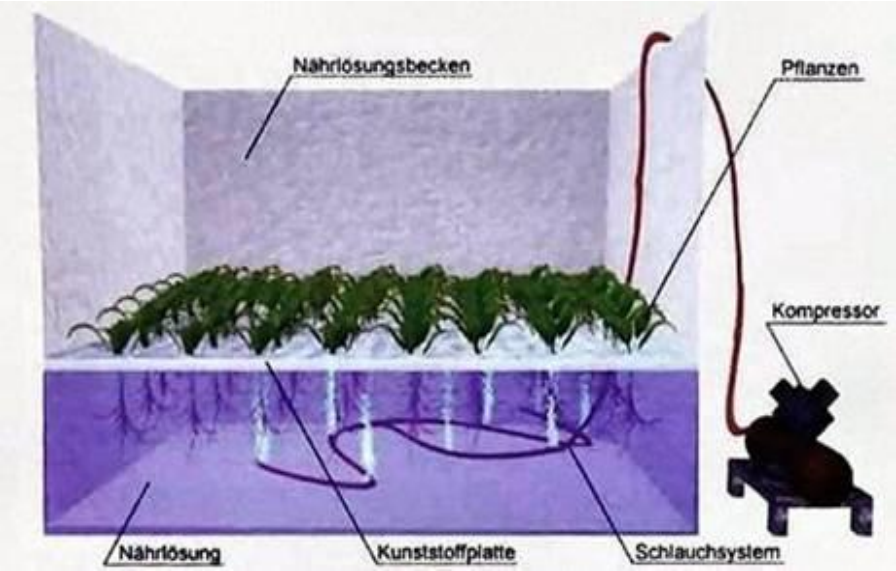
Smetanska, 2005

Glucosinolates in cell cultures and exudates of *Sinapis alba*



Cai, Kastell, Smetanska, 2010

Extraction: exudates



"Green chemistry"

Rapeseed

milling

Rapeseed meal

enzyme inactivation

Homogenised slurry

centrifugation, separation

Enzyme reactor

cell wall degrading enzymes

Oil, Hulls, Emulsion

ultrafiltration/
reverse osmosis

Exudates

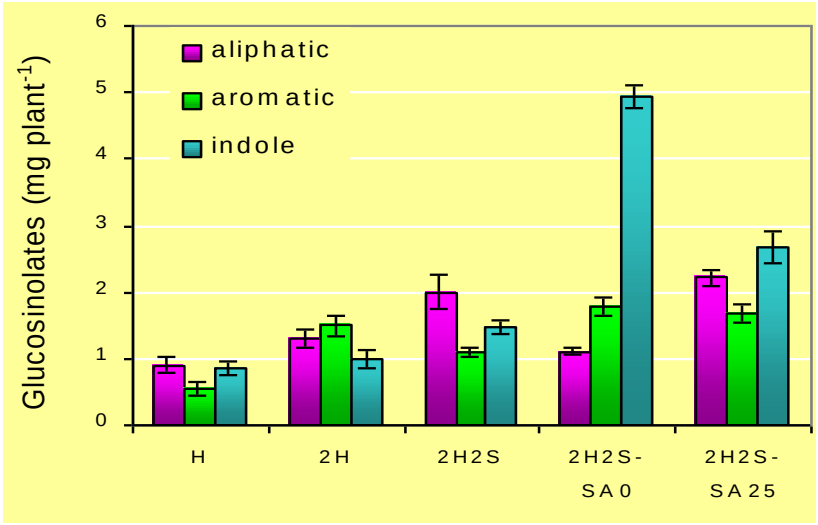
proteins, lipids, biocides, (4th generation glucosinolates)

Smetanska, Schreiner, Schonhof, Krumbein, Knorr
Patent 10 2006 004 829.6/42, PCT/EP 2007/000788

Smetanska et al, 2006; Leibnitz Institute of Vegetable and Ornamental crops – WG Schreiner

Extraction: exudates

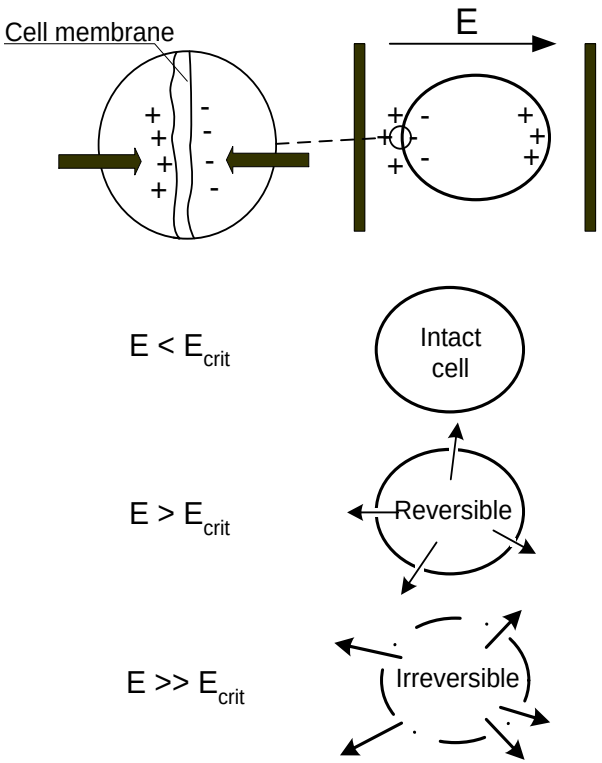
Glucosinolates in plant exudates of *Brassica rapa* (30 days)

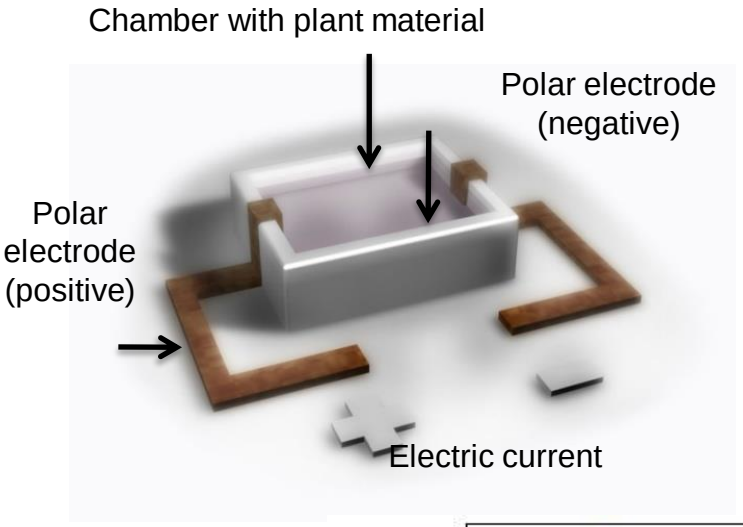


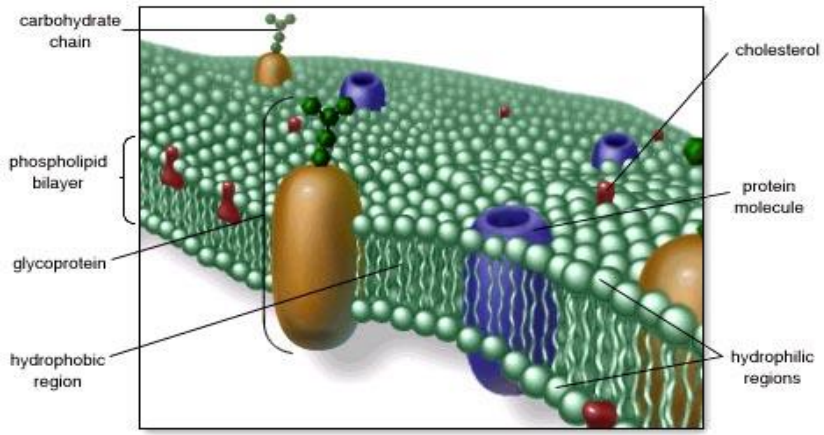
H – Hoagland solution, SA – salicylic acid

Schreiner, Krumbein, Knorr, Smetanska, 2010;

Extraction: Pulsed Electric Fields







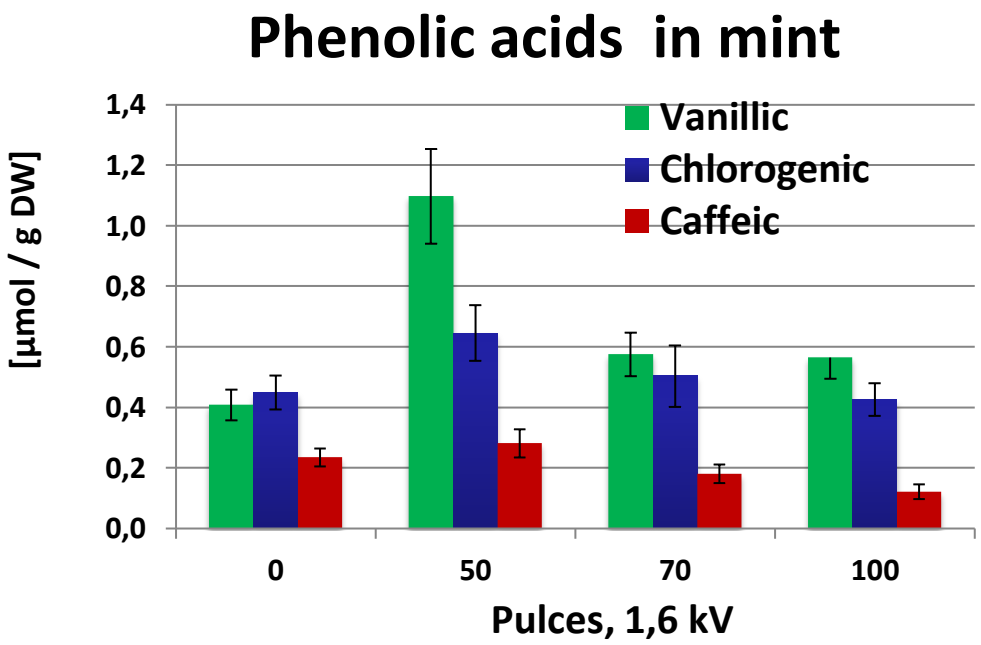
Pulsed electric field (PEF) processing is a method for processing cells by means of brief pulses of a strong electric field. PEF holds potential as a type of low temperature alternative pasteurization process for sterilizing food products. In PEF processing, a substance is placed between two electrodes, then the pulsed electric field is applied.

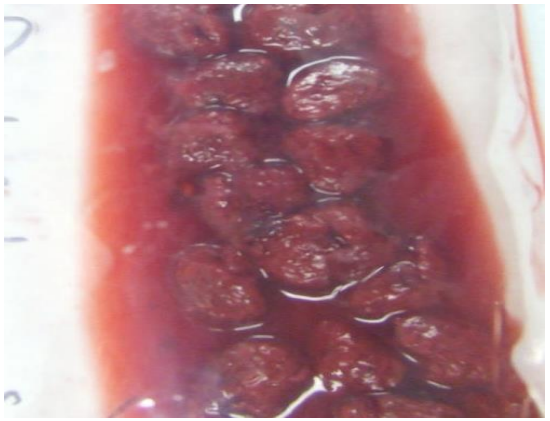
Extraktion: Pulsed Electric Fields

Electroporation through high field electric pulses

Destabilization of cell membranes
Electric field - kV/cm for ns -µs.

The electric field enlarges the pores of the cell membranes which kills the cells and releases their contents. PEF for food processing is a developing technology still being researched.

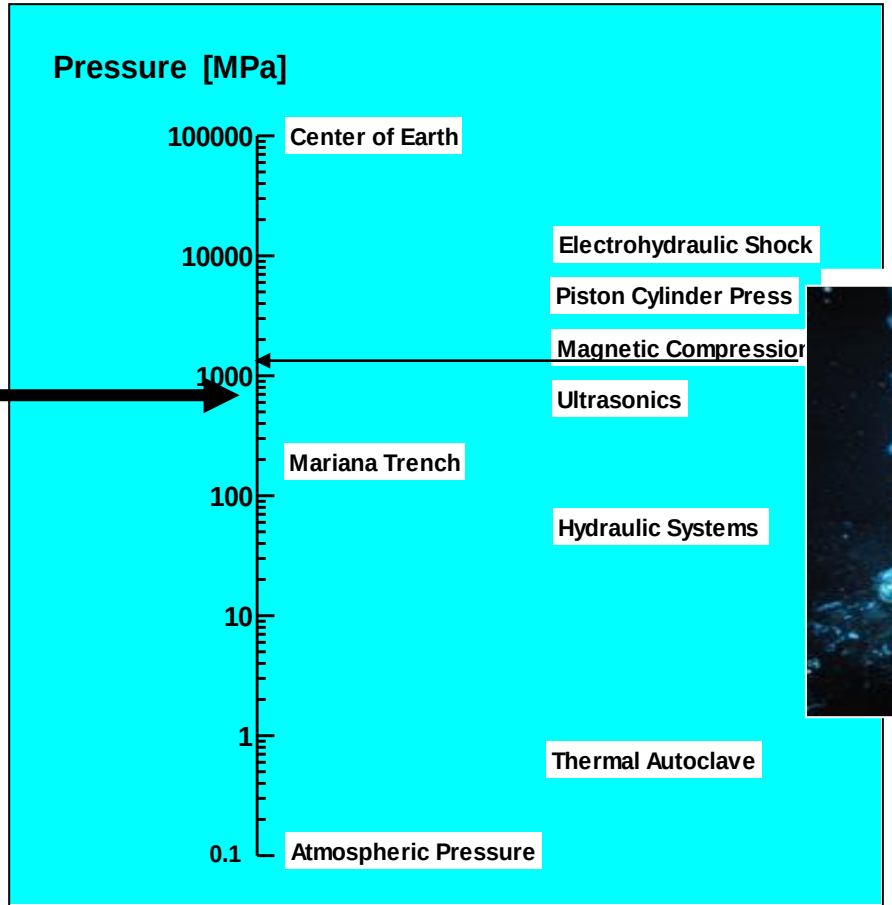


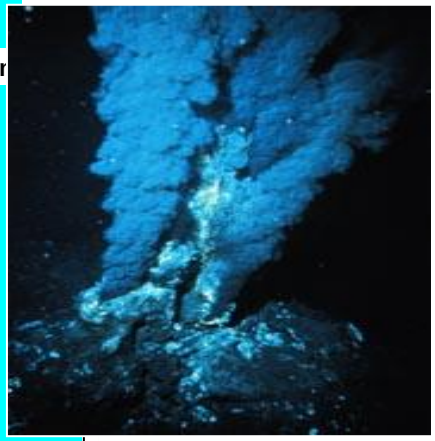


Extraction: High hydrostatic pressure

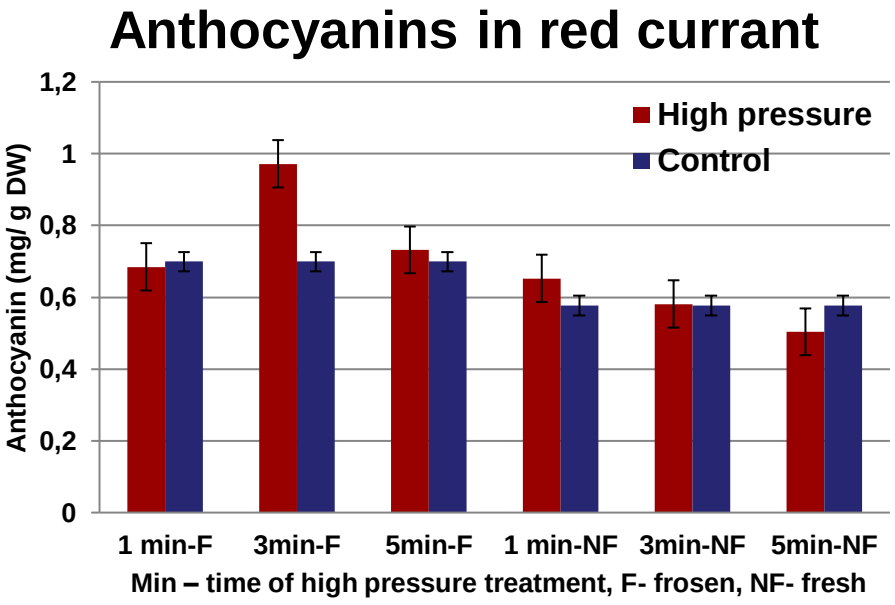
High pressure food preservation

Pressed inside a vessel exerting 70,000 pounds per square inch or more, food can be processed so that it retains its fresh appearance, flavour, texture and nutrients while disabling harmful microorganisms and slowing spoilage.





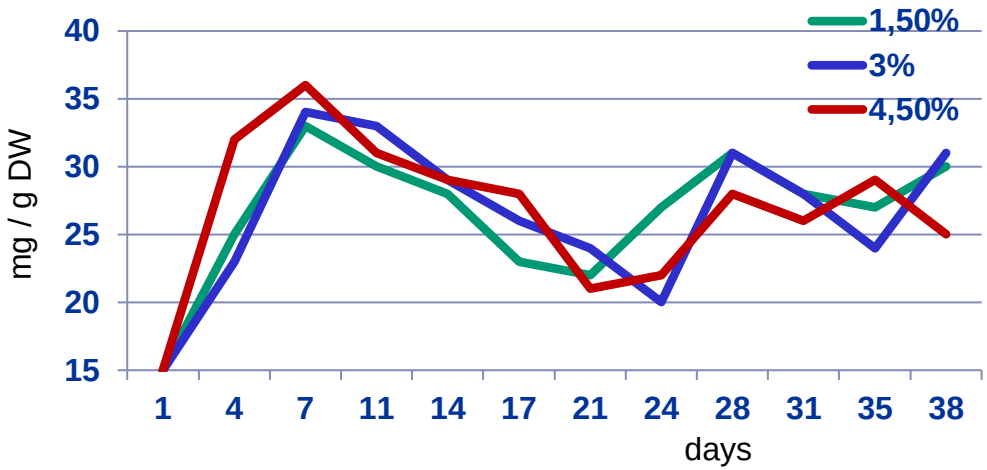
Extraction: High hydrostatic pressure



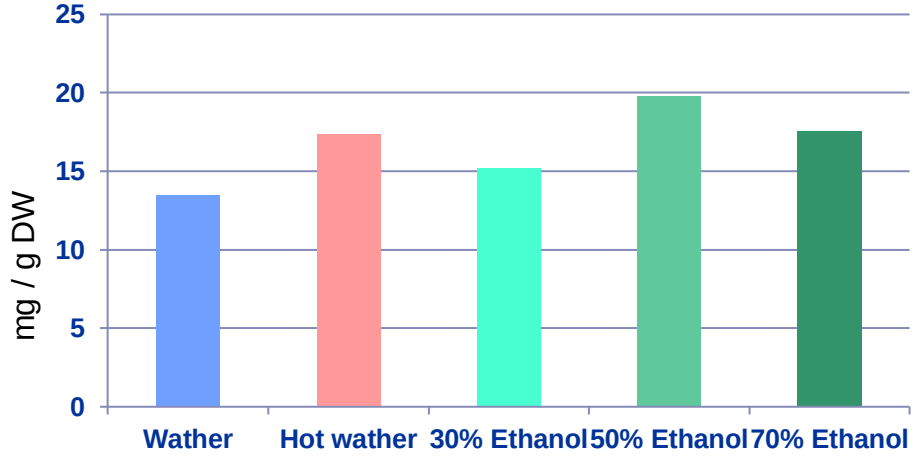
* 50 KPa

Extraction: Fermentation

Anthocyanin content in fermented red cabbage under different salt concentrations



Anthocyanin content in solvent extracts of red cabbage



Fermentation

Encapsulation

Extraction

Microbiology and genetics – WG Stahl; Benz, DAAD - Hunaefi, Ravichandran, Saw, Smetanska

Anti-oxidative activity

- **Antioxidants**
  - **Enzymatic**
    - Primary enzymes
      - *Superoxide dismutase (SOD)*
      - *Catalase*
      - *Glutathione peroxidase*
    - Secondary enzymes
      - *Glutathione reductase*
      - *Glucose-6-phosphatase-dihydrogenase*
  - **Non-enzymatic**
    - Cofactors
      - *Co-enzyme Q10*
    - Minerals
      - *Zinc, selenium*
    - Organosulfurcompounds
      - *Allyl sulfide indoles*
      - *glutathione*
    - Vitamins and derrivatives
      - *A, C, E, K*
    - Carotenoids
      - *Beta caroten*
      - *Lutein*
      - *Zeaxanthin*
    - Nitrogen non protein compoiunds
      - *Uric acid*
    - Flavonoids
      - Flavonols
        - *Quercetin*
        - *Kaempferol*
        - Isoflavonoids
          - *Genistein*
      - Flavanols
        - *Catechin*
        - Flavonones
          - *Hesperidin*
      - Anthocyanins
        - *Cyanidin*
        - Flavones
          - *Chrysin*
    - Phenolic acids
      - Hydroxybenzoic acids
        - *Ferulic acid*
        - *p-coumaric acid*
      - Hydroxycinnamic acids
        - *Gallic acid*

Anti-oxidative activity

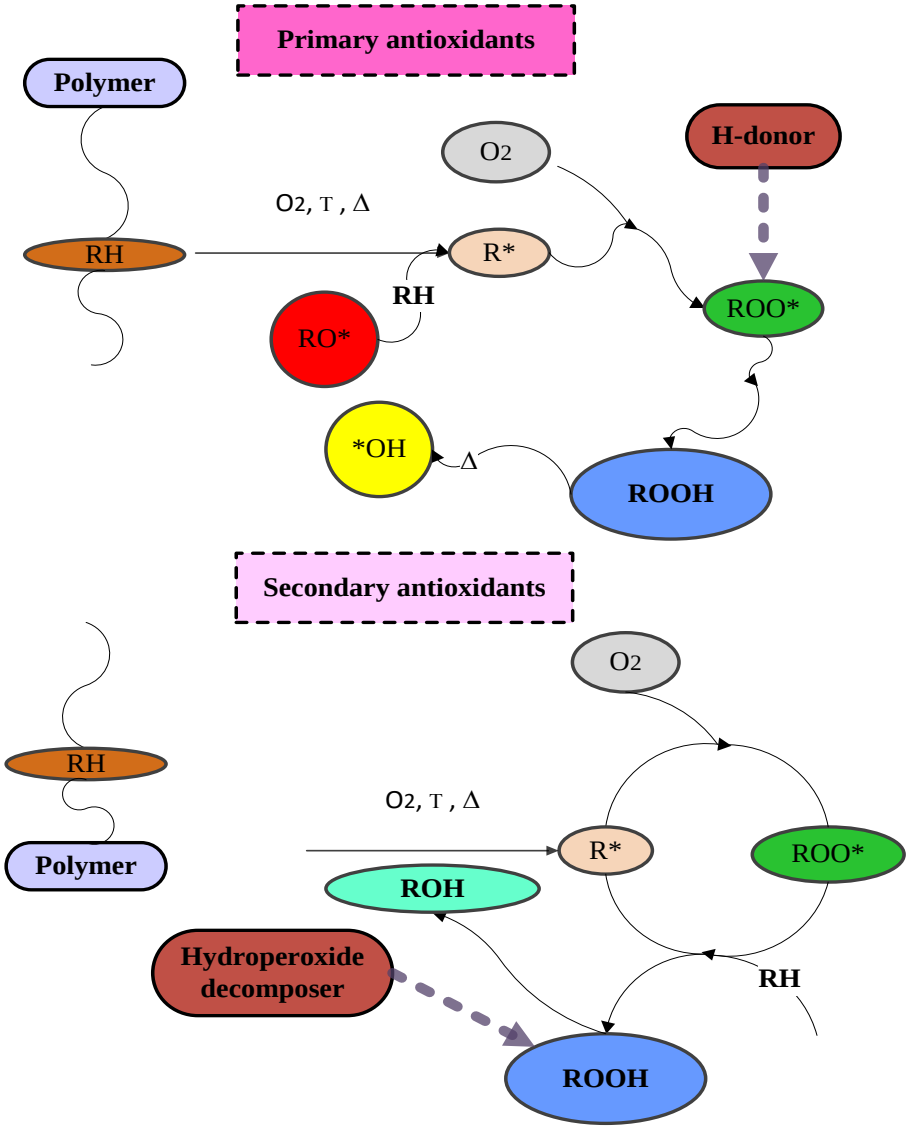
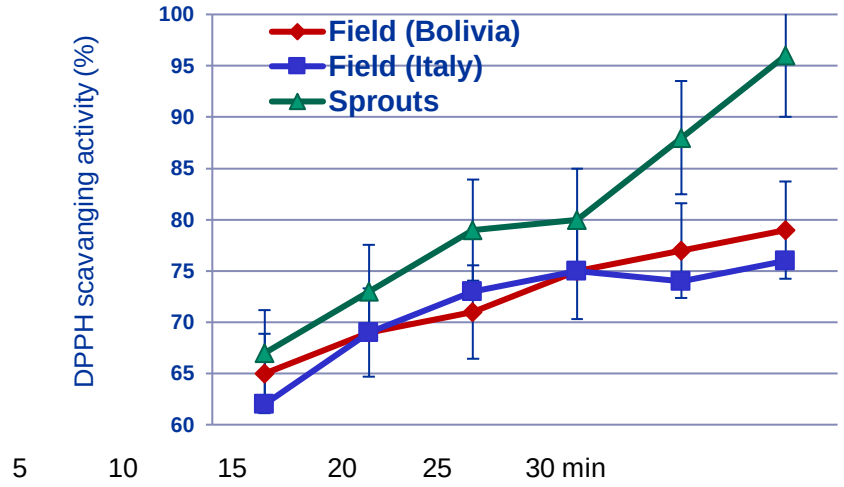


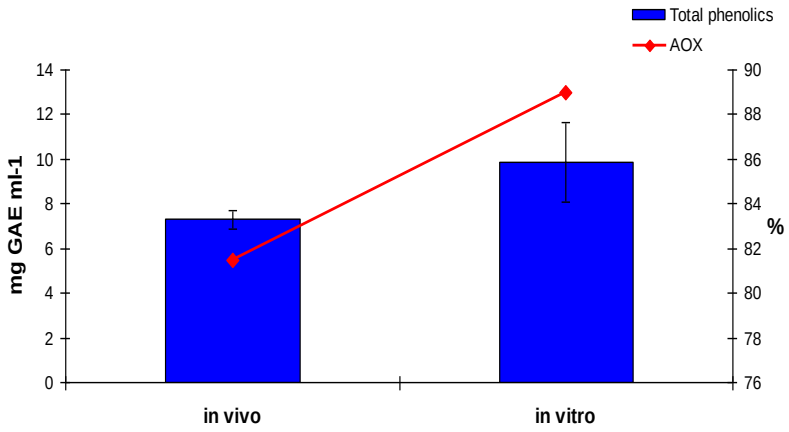
Illustration mechanism of primary and secondary antioxidants

Anti-oxidative activity

Free radical scavenging activity of Stevia

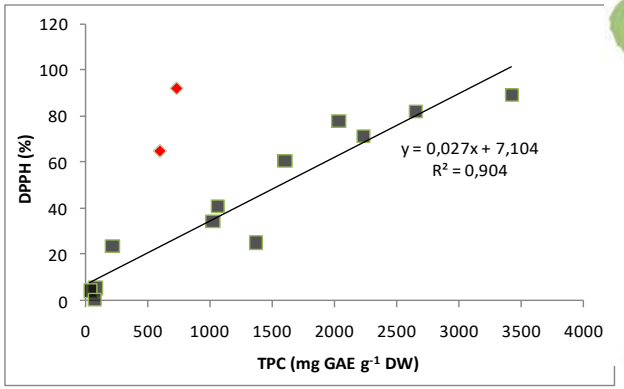
1,1-diphenyl-2-picrylhydrazyl (DPPH)





Correlation between DPPH radical scavenging activity (%) and total phenolic content (TPC) (mg GAE g-1 DW) of buckwheat sprouts *in vivo* and *in vitro*

Correlation between DPPH radical scavengin activity (%) and total phenolic content (TPC (mg GAE g^{-1} DW) in vitro Stevia sprou cultures



BPM, Channel - Schevchenko, Wendt, Mohdaly, Sytar, Smetanska

Vegetative seeds

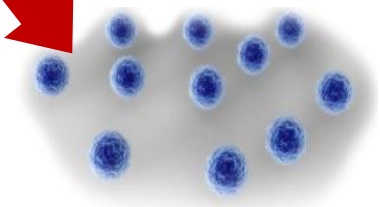
Encapsulated somatic Embryos

plant

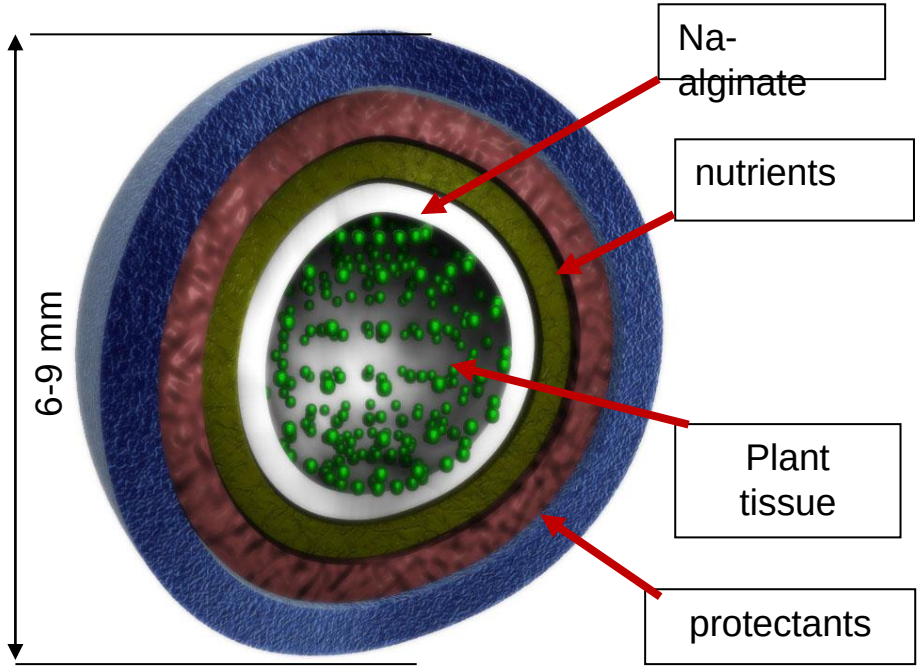
sprout culture

bioreactor

encapsulation

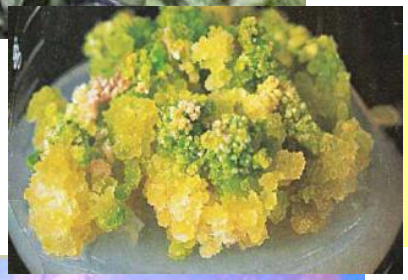


vegetative seeds



Milestones :

Screening of plants for secondary metabolite content



Establishment of shoot, hairy root and cell cultures

Comparison of different plants' systems with exudates and by-products

Biosynthesis targeting by nutrient supply, phytohormons, precursors, and elicitors

Development of extraction technology (solvents, membrane permeabilization).