Assimilate partitioning in higher plants
and
Plant Phenomics

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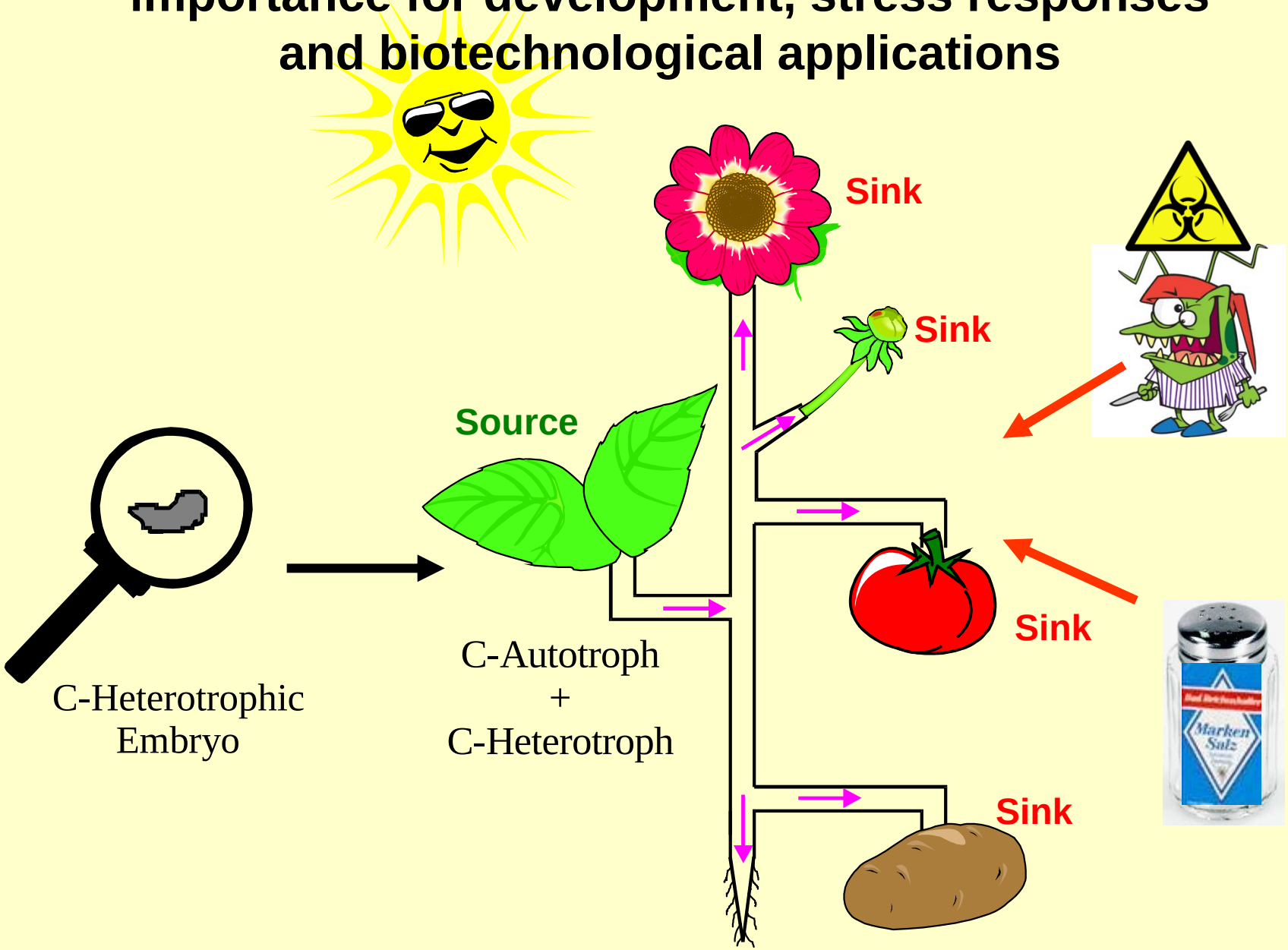
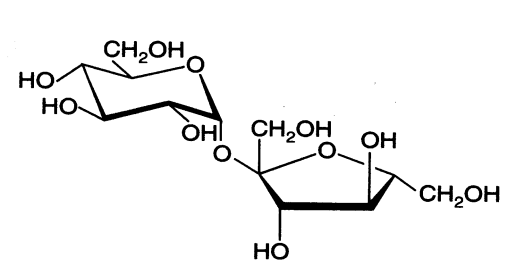
Assimilate partitioning in higher plants: importance for development, stress responses and biotechnological applications



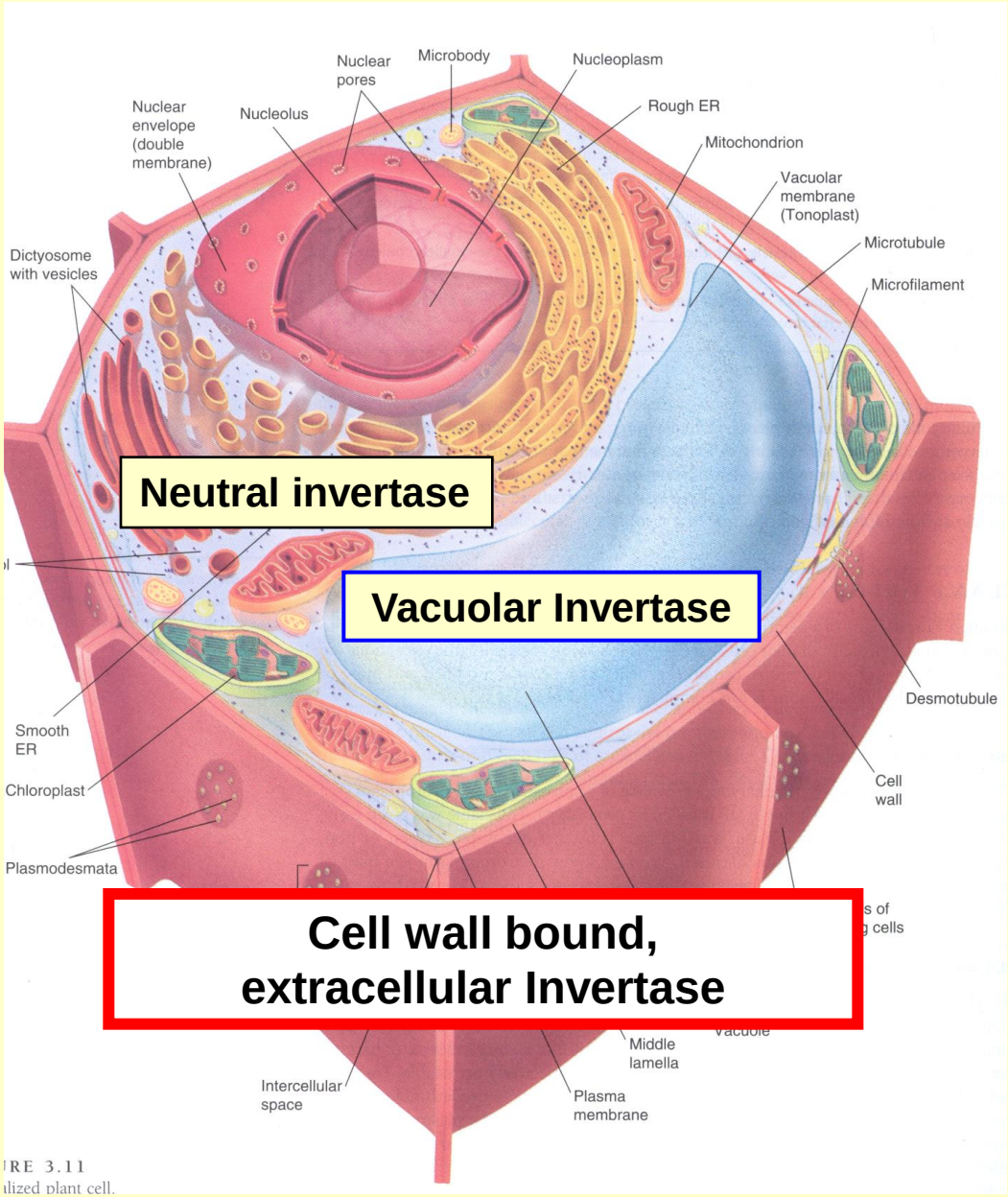
The Invertase Isoenzymes



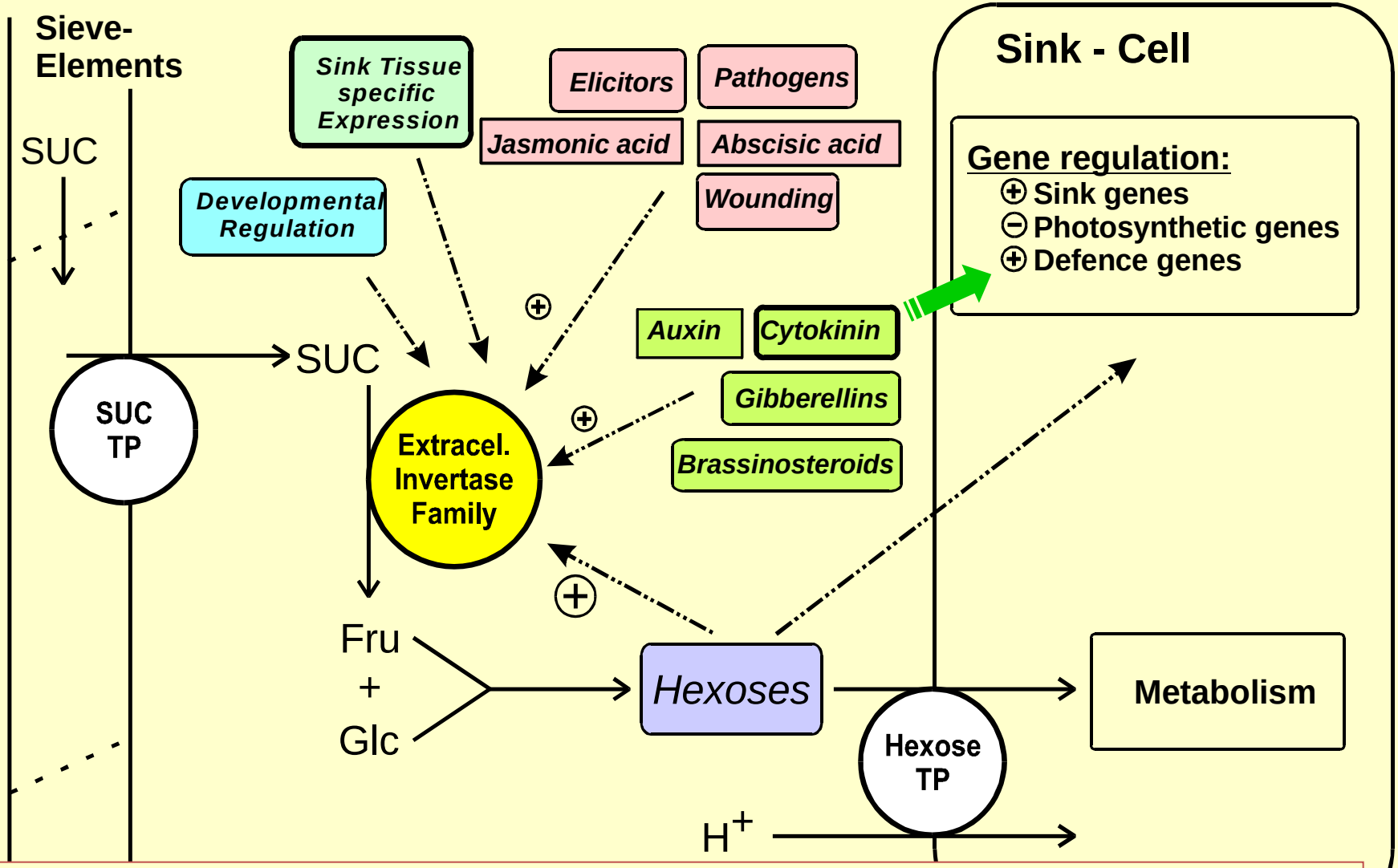
Sucrose

α-D-Glucopyranosyl-ß-D-fructofuranoside

Glucose + Fructose



Extracellular Invertase: Key metabolic Enzyme and PR Protein

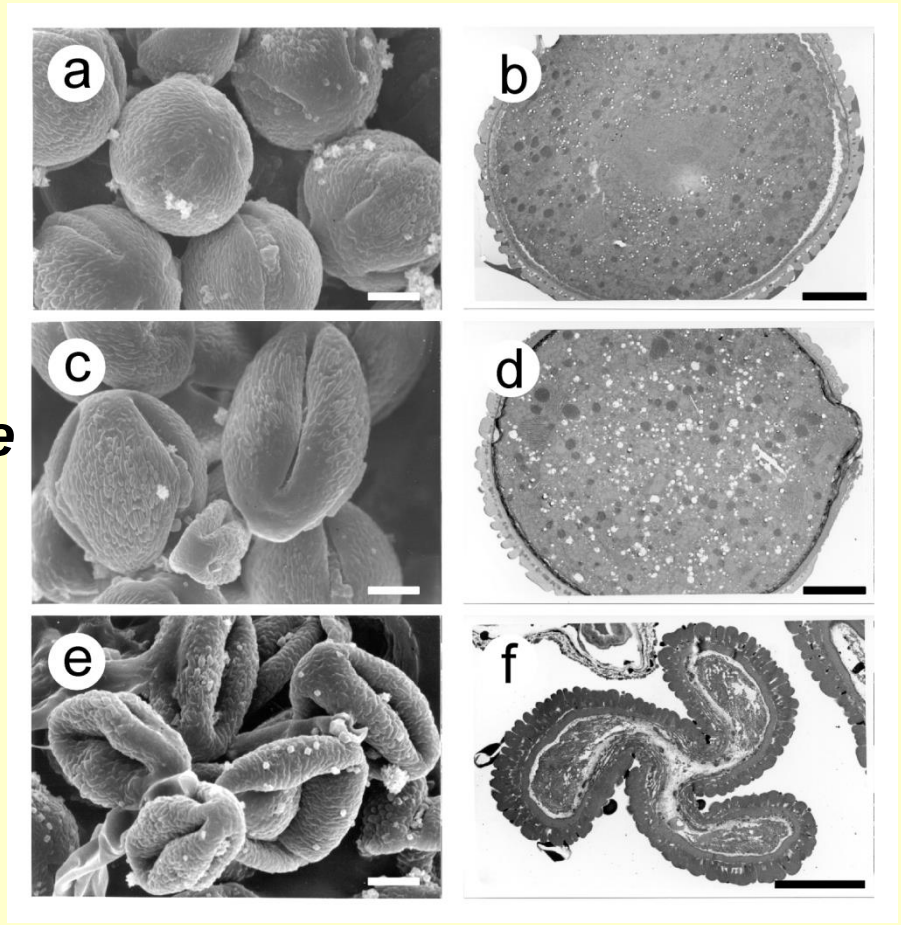


Roitsch (1999) COPS 2, 98f; Roitsch et al. (2004) TIPS 9, 607f; Berger et al. (2007) JXB 58, 4019f
Albacete et al. (2011) Phyton 50,181; Großkinsky et al. (2012) Plant Sci. 195, 54f

Extracellular invertase is essential for pollen development

Antisense-Repression of Extracellular Invertase Nin88 results in an Arrest of Pollen Development and male sterile Plants

SEM (2700x) TEM (5600x)

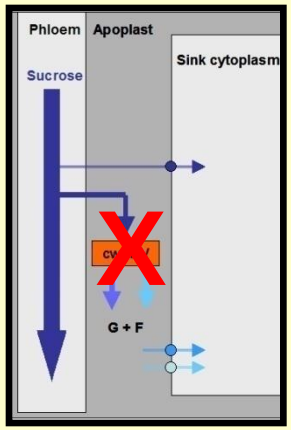


Wildtype

NT23-17

NT23-59

Nin88::
Nin88-Antisense



Goetz et al. (2001) PNAS

Relation between phytohormone action and carbohydrate metabolism

Extracellular Invertase is essential for the delay of Senescence by cytokinins

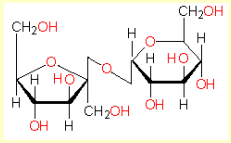
Cytokinin ➔ Extracellular Invertase ➔ Delay of Senescence

SAG12:
Cin1

W 38

Balibrea et al..
(2004) Plant Cell

Co-ordinated regulation of source/sink relations and defence response by sugars and stress related stimuli



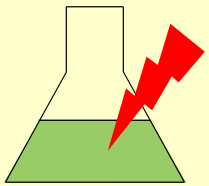
Sucrose

Stress/Pathogen related stimuli

Calcium-Signatures

Glucose

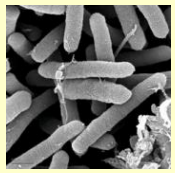
Stauro-sporin



MAP Kinases
Points of signal integration and distribution

Induction

Repression



Sink-specific genes

Defence related genes

Photo-synthetic genes

Ts-Factors
Hsf
WRKY
BZip

Extracellular Invertase

PAL

RbcS

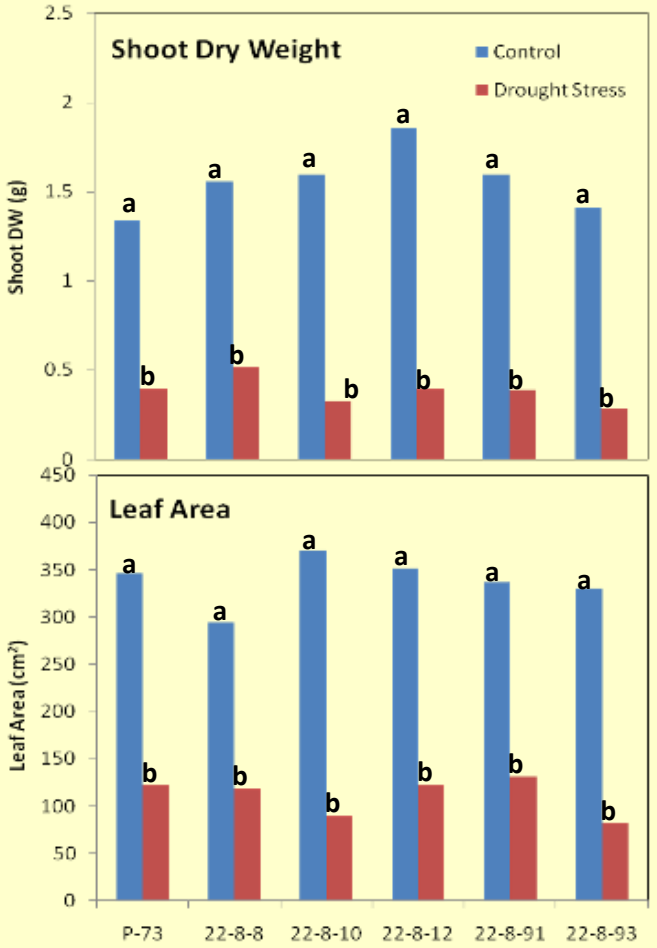
Roitsch (1999) COPS 2: 198-206
Ehness et al. (1997) Plant Cell 9: 1825

Ectopic expression of Extracellular invertase results in extreme drought stress tolerance

WT

CIN1 1-91

Drought stress tolerance in *CIN1* is not accomanied by a yield penalty under control conditions



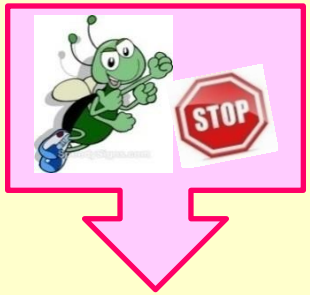
Cytokinins stimulate immunity via stimulation of phytoalexin synthesis within the Phytohormones Networks

Phytohormone defence backbone:

- Salicylic Acid
- Jasmonic Acid
- Ethylene

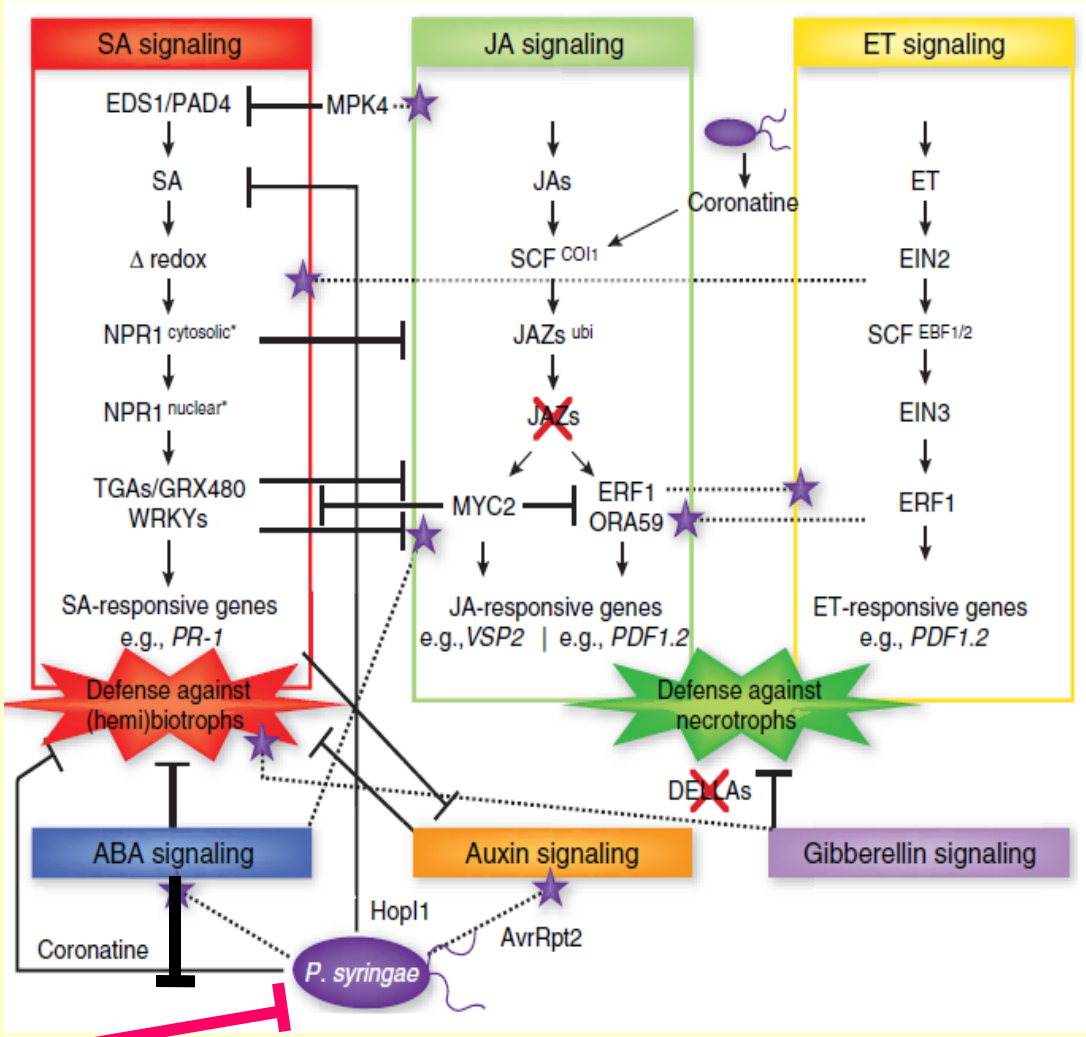
Additional interactions :

- Abscisic Acid
- Auxin
- Gibberellin



+ Cytokinin signalling

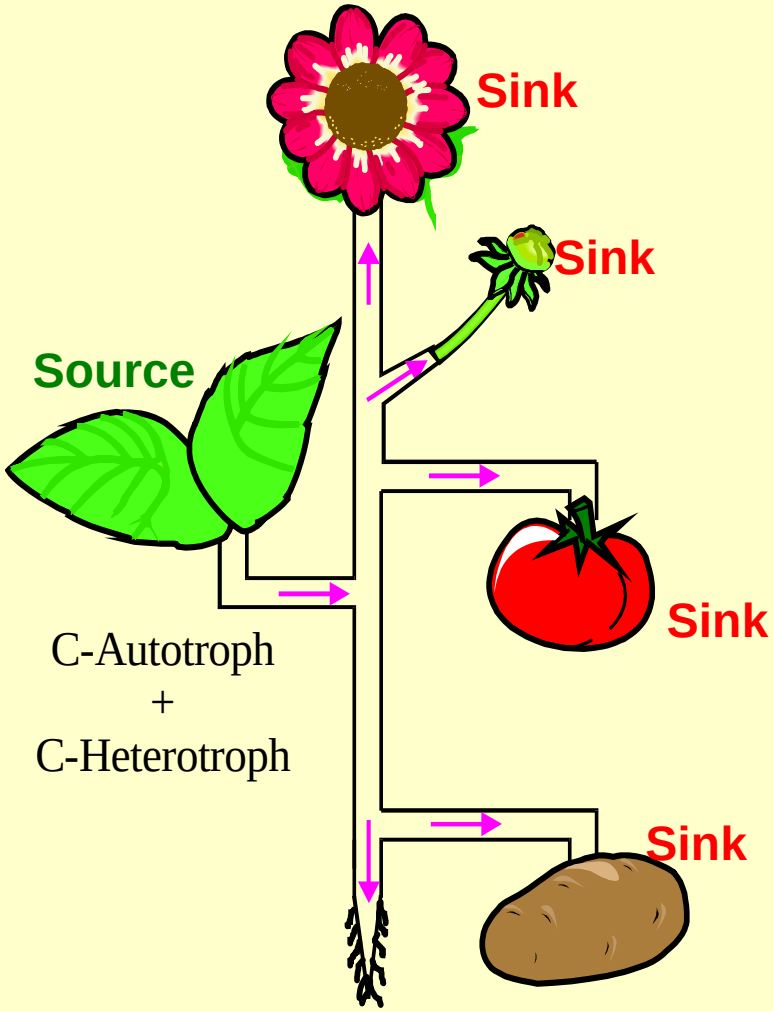
Phytoalexins



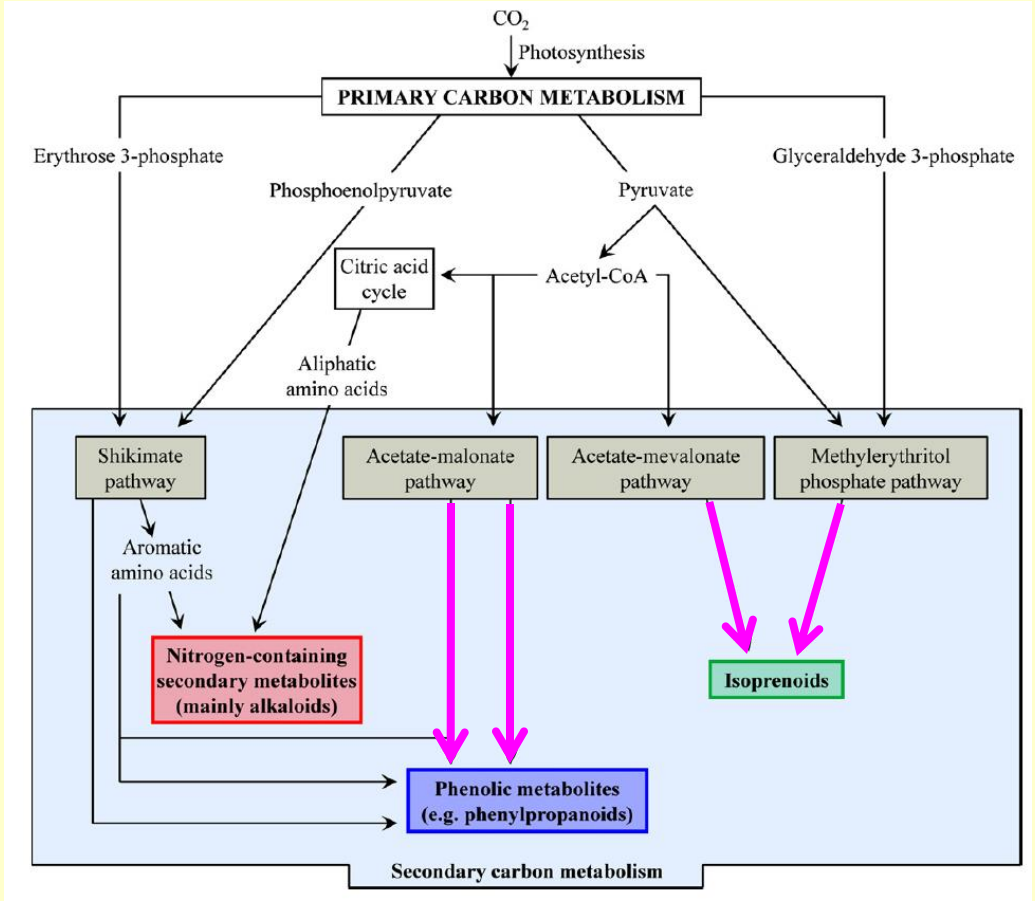
Pieterse et al. (2009), *Nature Chemical Biology*

Different scales of carbohydrate partitioning

(A) Long distance transport from source and sink tissues



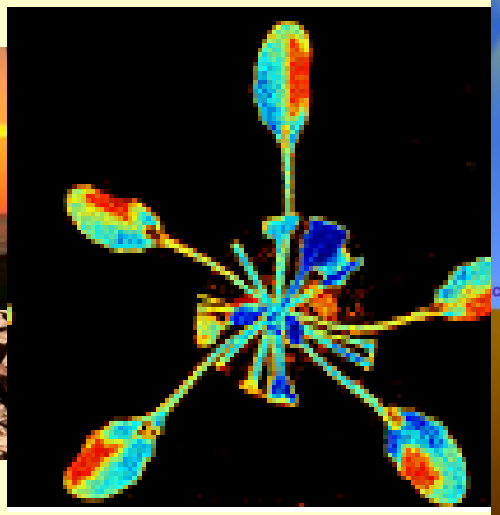
(B) Metabolic channeling from primary to secondary metabolism

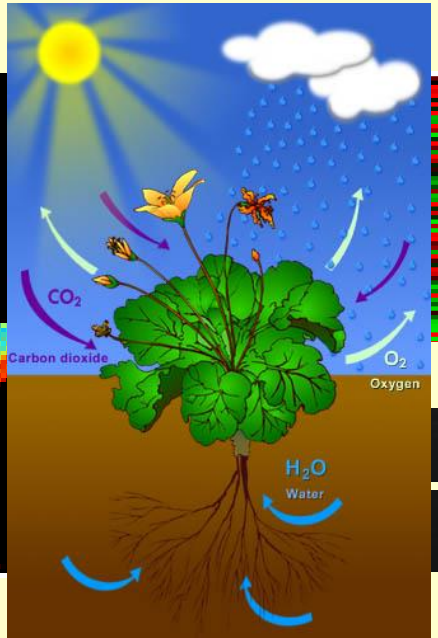


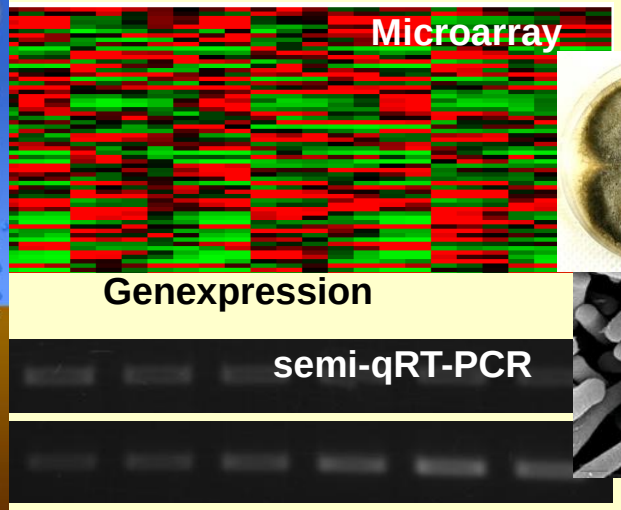
Phenomics

Development and application of high-thoughput, multi-dimensional and dynamic phenotyping

- Gene to phenotype modelling -

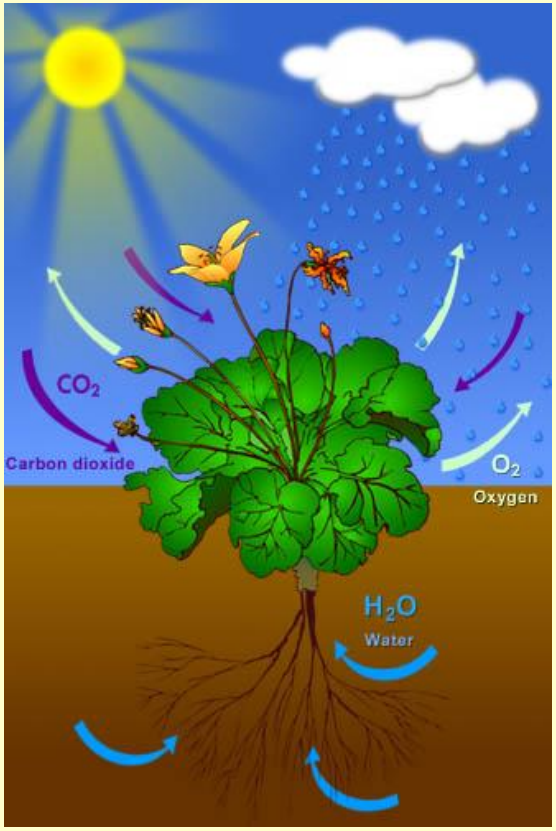






Phenotypic variation

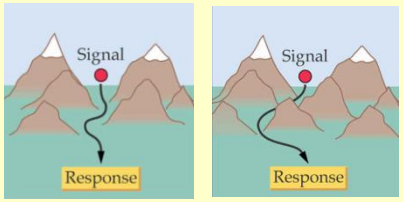
… is produced through a complex web of interactions between
Genotypes x Environment x Management

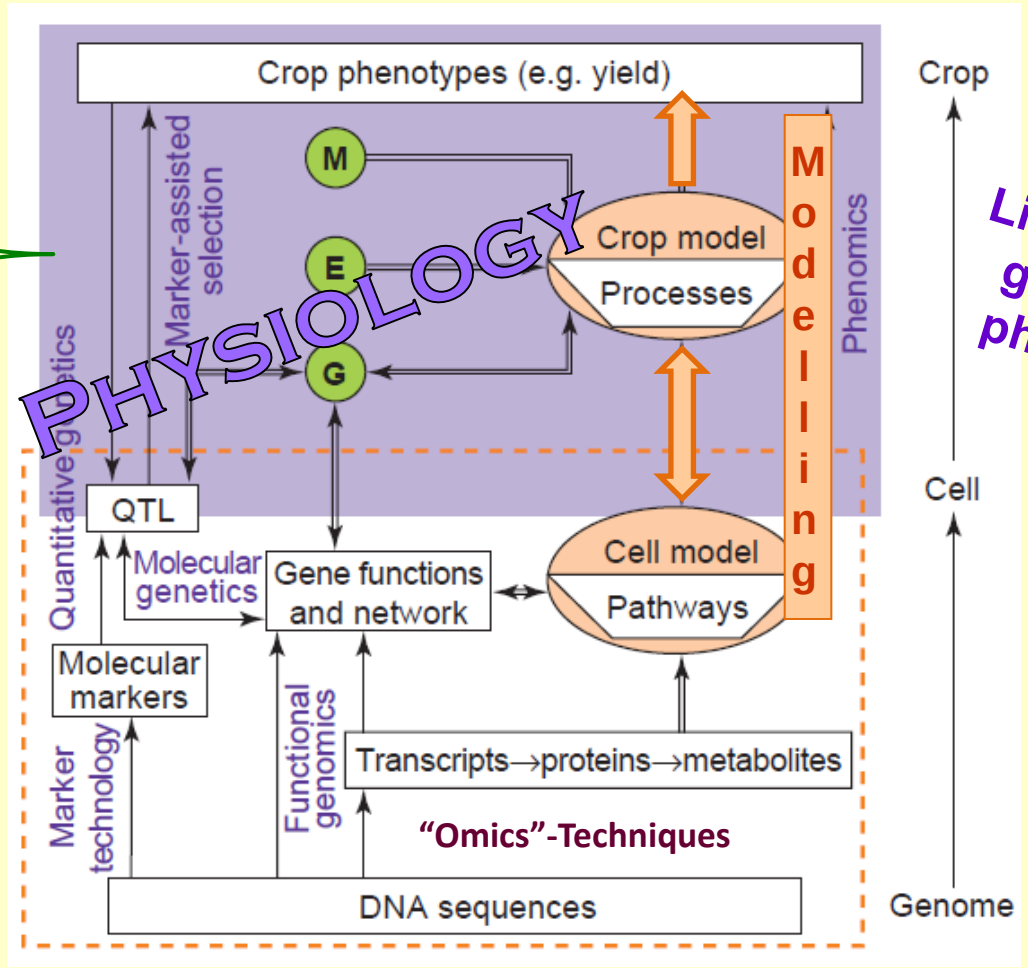


Plant Physiology – Key Interface between genome and quantitative traits that determines harvest yield and quality

Integration of

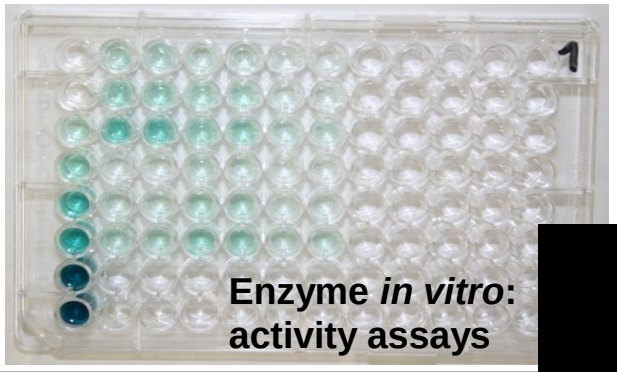
- **M**anagement actions
- **E**nvironmental variables
- **G**enetic coefficients





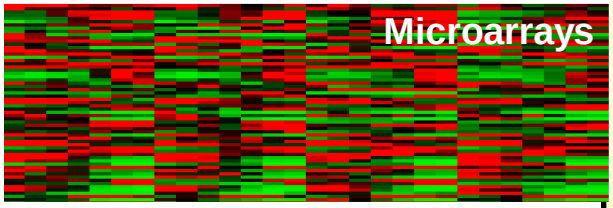
Yin, Struik & Kropff (2004) TIPS 9, 426-432. modified

Integration of non-invasive signatures with physiological phenotyping: „Plant physiology meets biophysics“



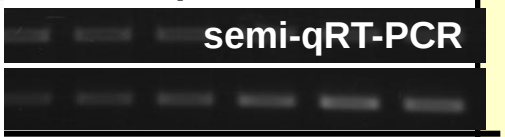
Enzyme *in vitro*:
activity assays

Enzyme *in situ:*
histochem. Aktivtätsnachweise

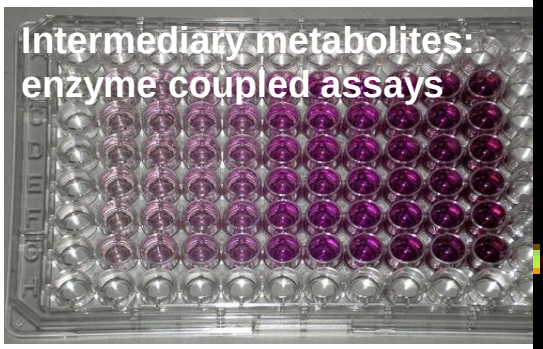


Microarrays

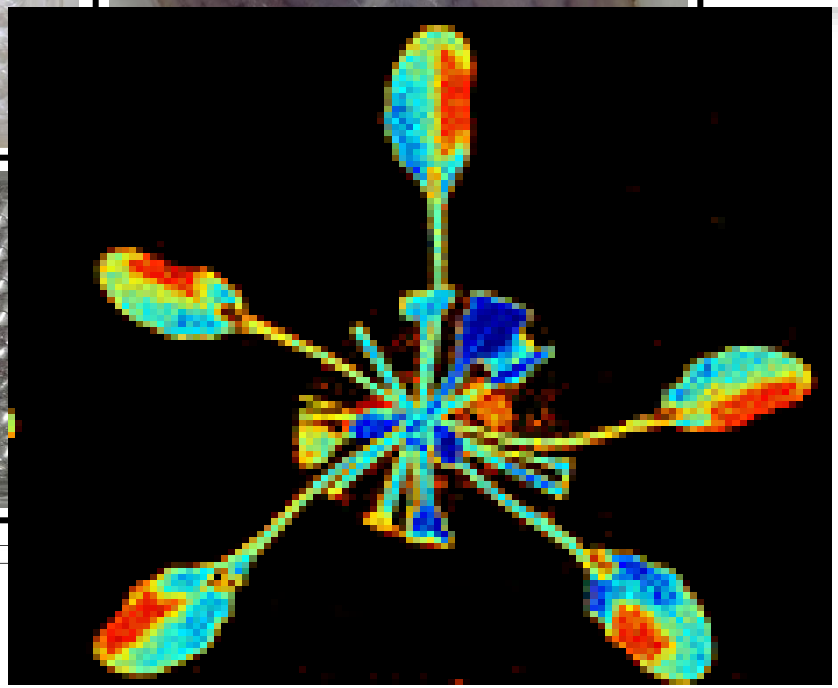
Genexpression



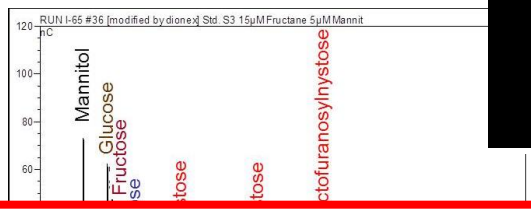
semi-qRT-PCR

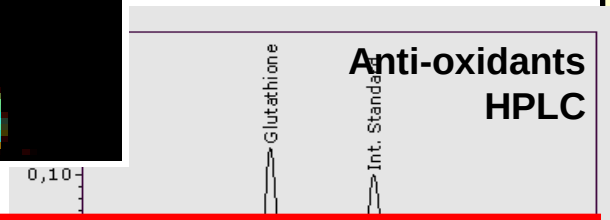


Intermediary metabolites:
enzyme coupled assays



Phytohormones:
U-HPLC-HESI-MS/MS



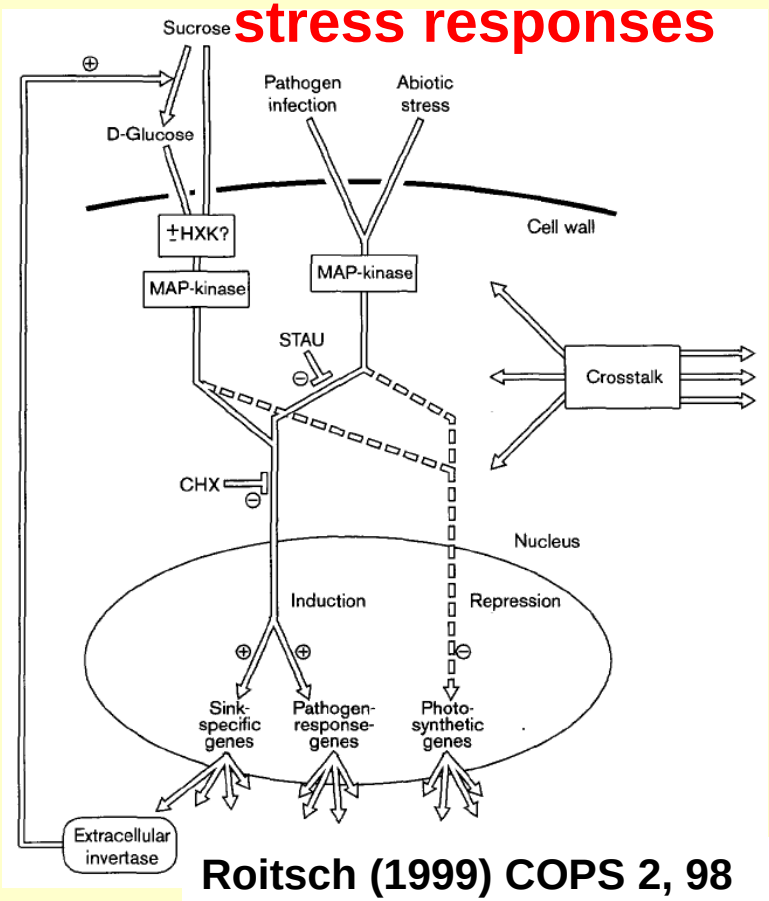


Anti-oxidants
HPLC

Linking hyper-scale, non-invasive imaging to temporal and spatial dynamics of physiological processes and DNA-based markers

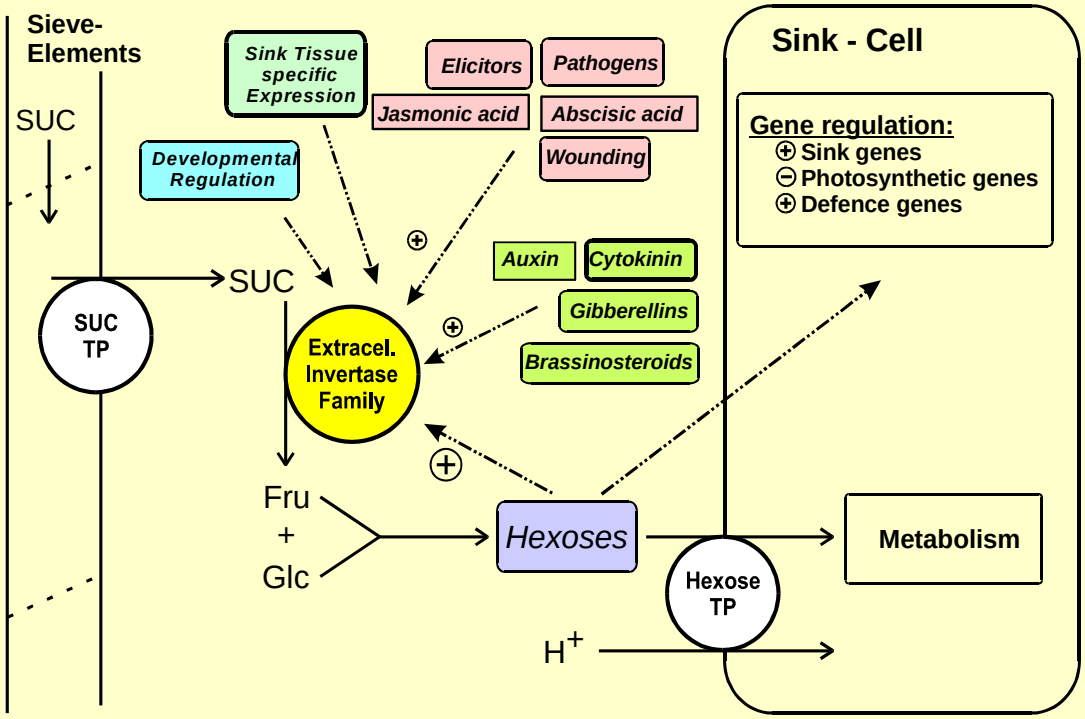
Development & stress responses are linked to plant carbohydrate metabolism

Co-ordinated regulation of source-sink relations & stress responses



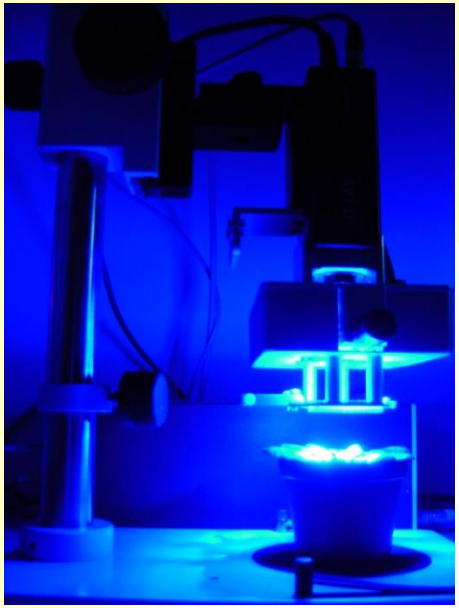
Roitsch (1999) COPS 2, 98
Berger et al. (2007) JXB 58, 4019
Albacete et al. (2011) Phyton 50,181

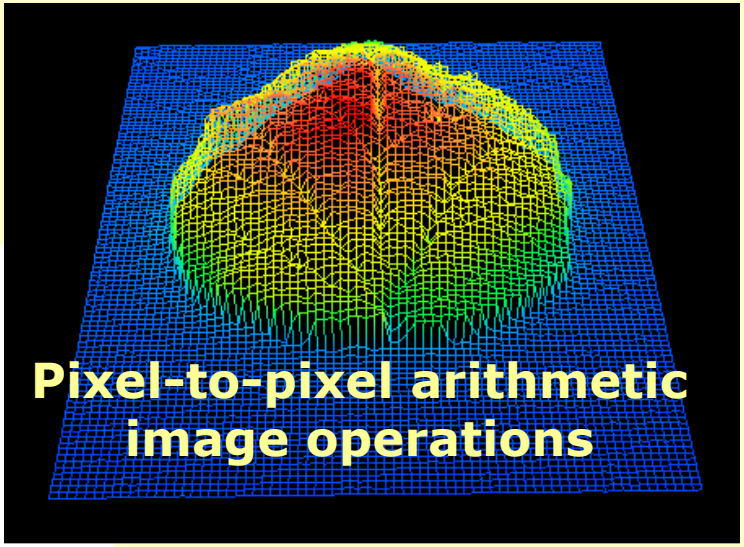
Extracellular Invertase: key metabolic enzyme and stress response protein

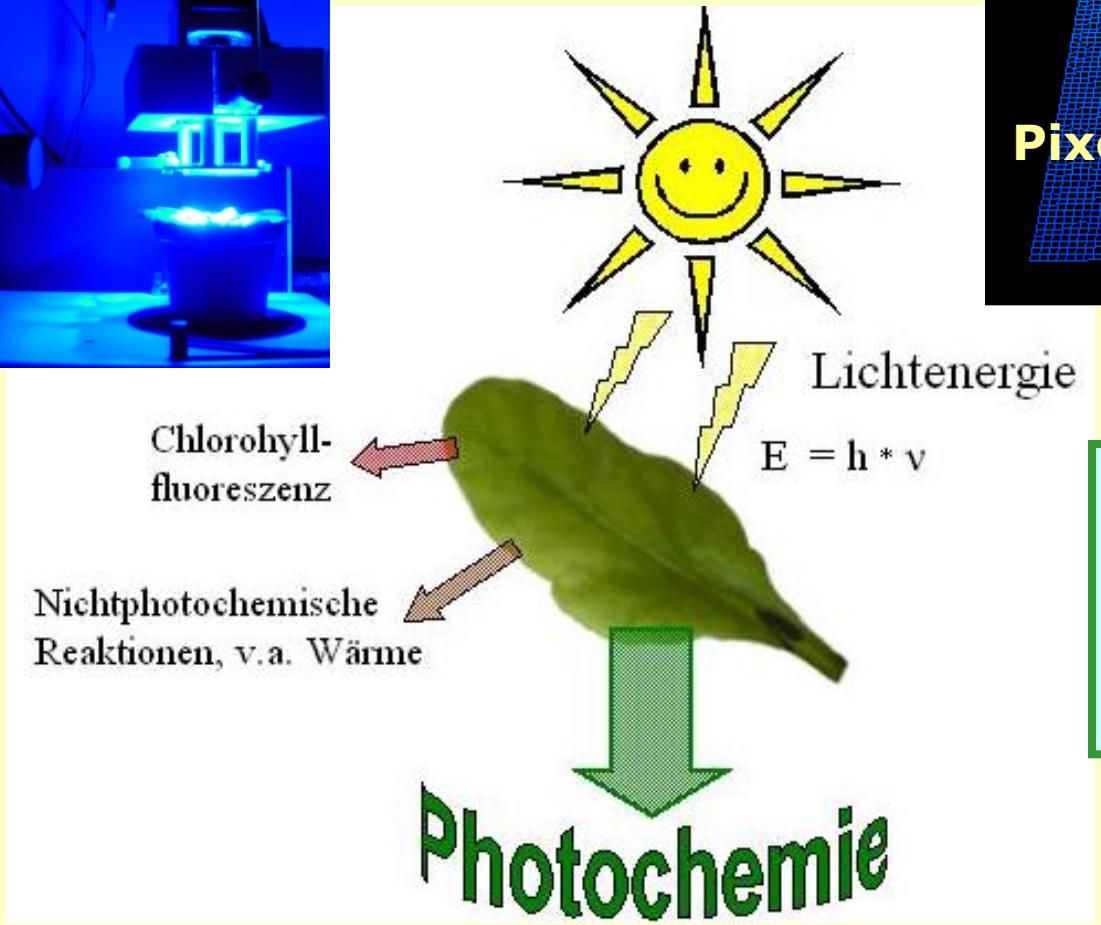


Roitsch et al. (1995) PlantPhys 108: 285
Roitsch et al. (2004) TIPS 9, 607
Albacete et al. (2011) Phyton 50,181

Chlorophyll Fluorescence Imaging



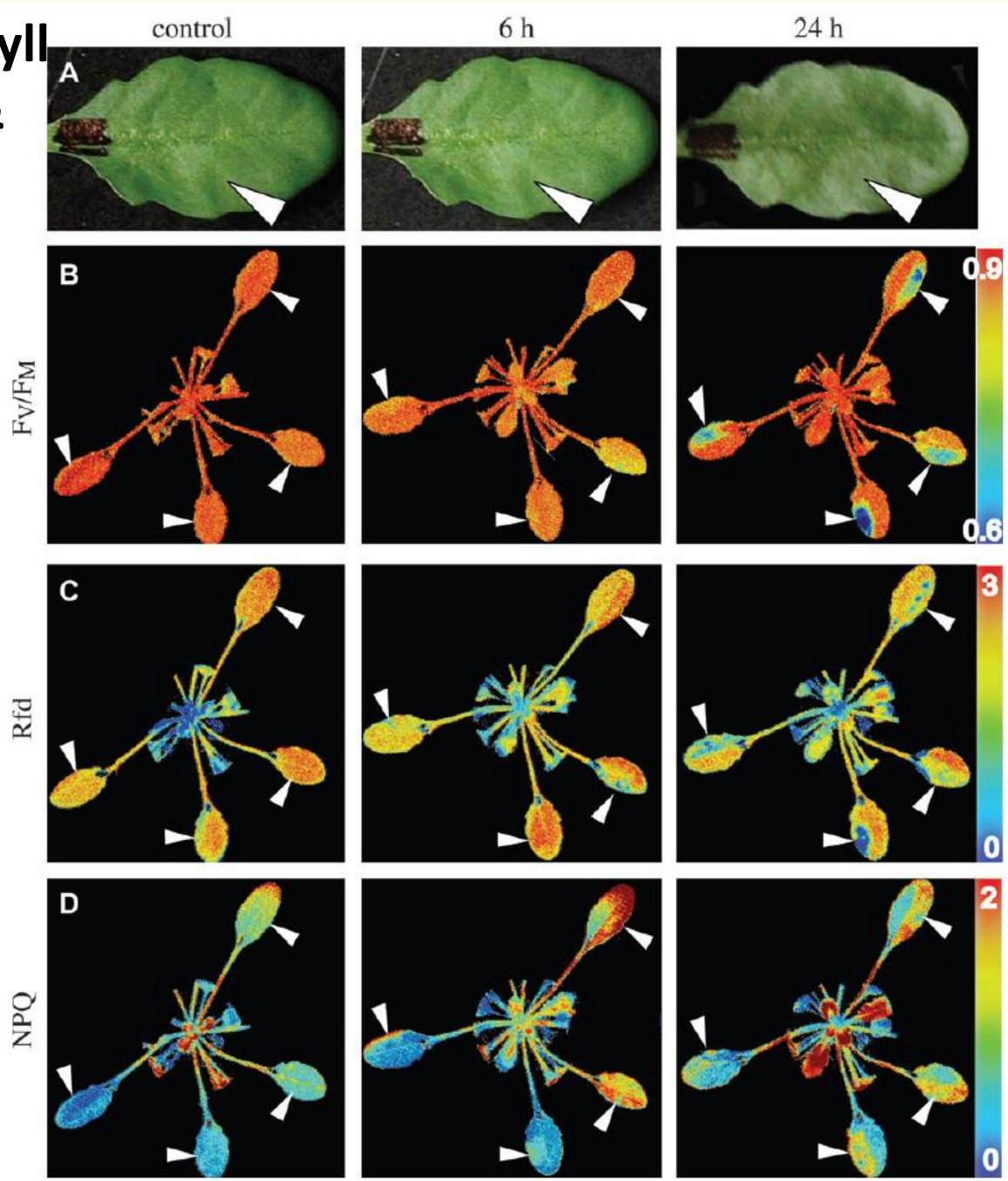




Advantages:

- Non invasive
- Very high sensitivity
- Spatial resolution

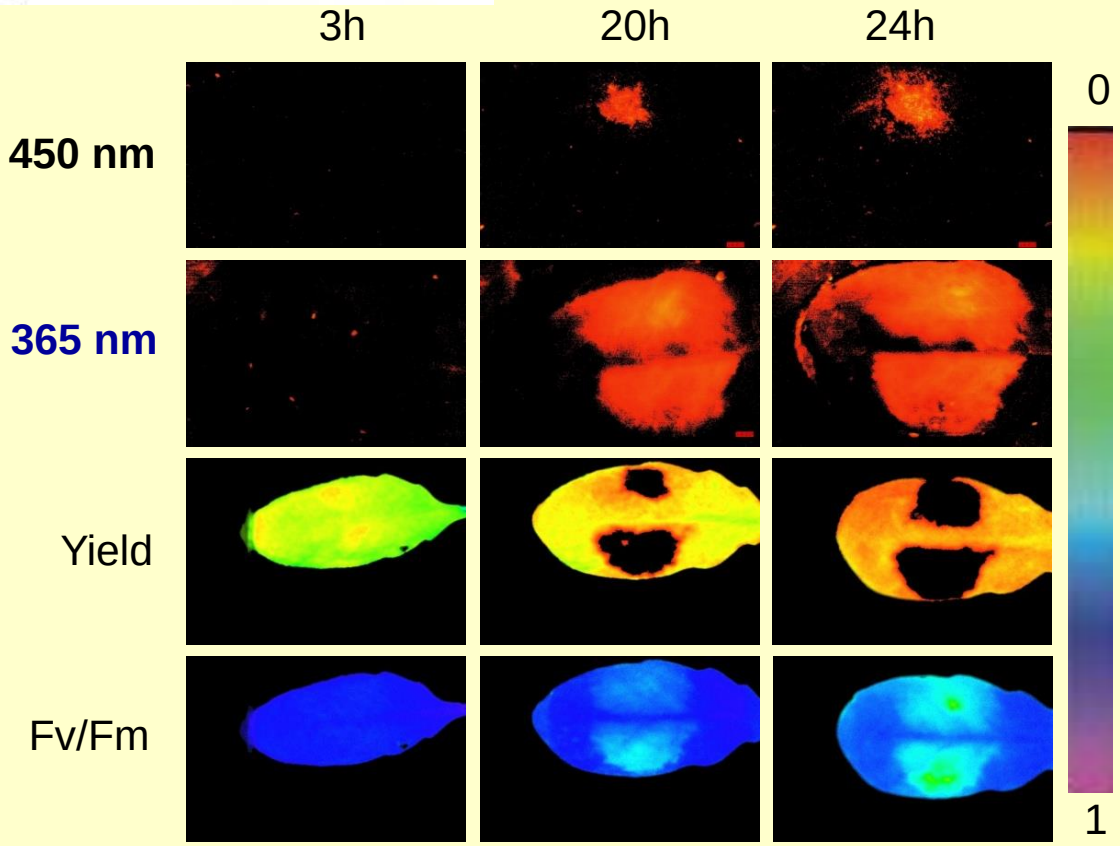
Combination of chlorophyll fluorescence imaging & statistical analysis: Combinatorial imaging



Berger (2007). J. Expt. 58, 797

Development of multifluorescence imaging

Discrimination between chlorophyll, phenolic and GFP fluorescence



Simultaneous infiltration of WT strain and GFP labelled strain of P. syringae

PAM/GFP- measuring head

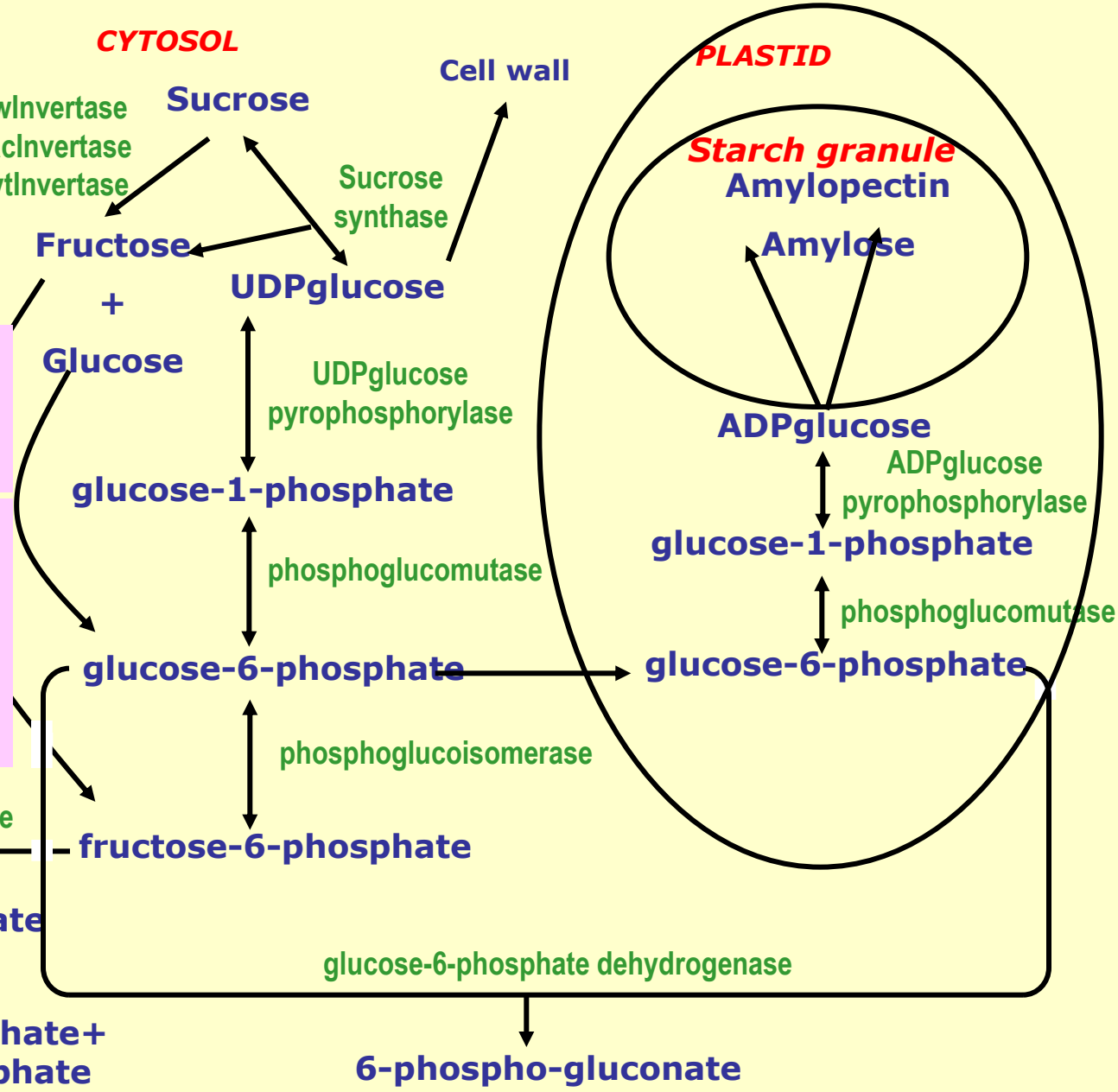
UV-light measuring head

PAM/GFP- measuring head

Enzyme activity signatures

Uniform extraction :
- small sample size
- direct comparison

96-well format and 3 plate readers:
- semi-high throughput
- 300 assays/day vs 30 in single cuvets



Characterisation of sugar beet development
Times course of enzyme activity signatures

Parameter [min/max]	No. 1	No. 2	No. 3	No. 4
Frischgewicht (0,01 – 25 g)	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲
Suc-Gehalt (10 – 60.000 µg/gFW)	▲ ▲ x ▲ ▲ ?	▲ ▲ x ▲ ▲ ?	▲ ▲ x ▲ ▲ ?	▲ ▲ x ▲ ▲ ?
Sucrol. Aktiv. ●	▼ ▼ ▼ ▼ ▼ ▼	▼ ▼ ▼ ▼ ▼ ▼	▼ ▼ ▼ ▼ ▼ ▼	▼ ▼ ▼ ▼ ▼ ▼
vacInv ●	▼ ▼ ▼ ▼ ▼ ▼	▼ ▼ ▼ ▼ ▼ ▼	▼ ▼ ▼ ▼ ▼ ▼	▼ ▼ ▼ ▼ ▼ ▼
cytInv ●	▼ ▼ ▼ ▼ ▼ ▼	▼ ▼ ▼ ▼ ▼ ▼	▼ ▼ ▼ ▼ ▼ ▼	▼ ▼ ▼ ▼ ▼ ▼
cwInv ●	▼ ▼ ▼ ▼ ▼ ▼	▼ ▼ ▼ ▼ ▼ ▼	▼ ▼ ▼ ▼ ▼ ▼	▼ ▼ ▼ ▼ ▼ ▼
SuSy (nd – 2 nkat*mg^{-1}Prot) ●	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ – ▲ ▲ ▲	▲ – – ▲ ▲ ▲	▲ – – ▲ ▲ ▲
PFK (nd – 1 nkat*mg^{-1}Prot) ●	▲ ▲ – ▲ ▲ ▲	▲ ▲ – ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲
UGPase (nd – 6 nkat*mg^{-1}Prot) ●	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲
PGI (nd – 20 nkat*mg^{-1}Prot) ●	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲
PGM (nd – 20 nkat*mg^{-1}Prot) ●	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲
G6PDH (nd – 3 nkat*mg^{-1}Prot) ●	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲	▲ ▲ ▲ ▲ ▲ ▲
Aldolase (< 0,1 – 2 nkat*mg^{-1}Prot) ●	∩ – ∩ ∩ ∩ ∩	∩ – ∩ ∩ ∩ ∩	∩ ∩ ∩ ∩ ∩ ∩	∩ ∩ ∩ ∩ ∩ ∩

▲ = Increase over time (90 days); ▼ = decrease over time; ∩ = transient; – = no change; x = n. d.; ? = no data yet

Instrumental analysis: Hormone levels

1. Cytokinins

a) biological active

- *trans*-Zeatin (tZ)
- *trans*-Zeatin-Ribosid (tZR)
- Dihydrozeatin (DHZ)
- Dihydrozeatin-Ribosid (DZR)
- Isopentenyl-Adenin (iP)
- *cis*-Zeatin (cZ)

b) conjugates:

- Isopentenyl-Adenin-7-Glucoside (IP7G)
- *trans*-Zeatin-O-Glucosid (ZOG)
- *trans*-Zeatin-O-Glucosid-Ribosid (ZOGR)
- *trans*-Zeatin-7-Glucoside (tZ7G)
- *trans*-Zeatin-9-Glucoside (tZ7G)

2) other hormones/ signal molecules

- **Indolacetic Acid (IAA)**
- **Abscisic Acid (ABA)**
- **Jasmonic Acid (JA)**
- **Salicylic Acid (SA)**

3) To be established: Gibberellins

- **G A1**
- **G A3**
- **G A4**
- **G A5**
- **G A6**
- **G A8**

UHPLC/Ms-Ms

Determination of spatial and temporal dynamics of plant responses during development within environmental constraints and interactions