Chl fluorescence signatures of plant functions: Application to study plant stress responses of primary carbohydrate metabolism

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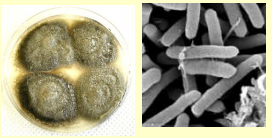
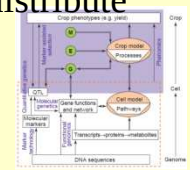
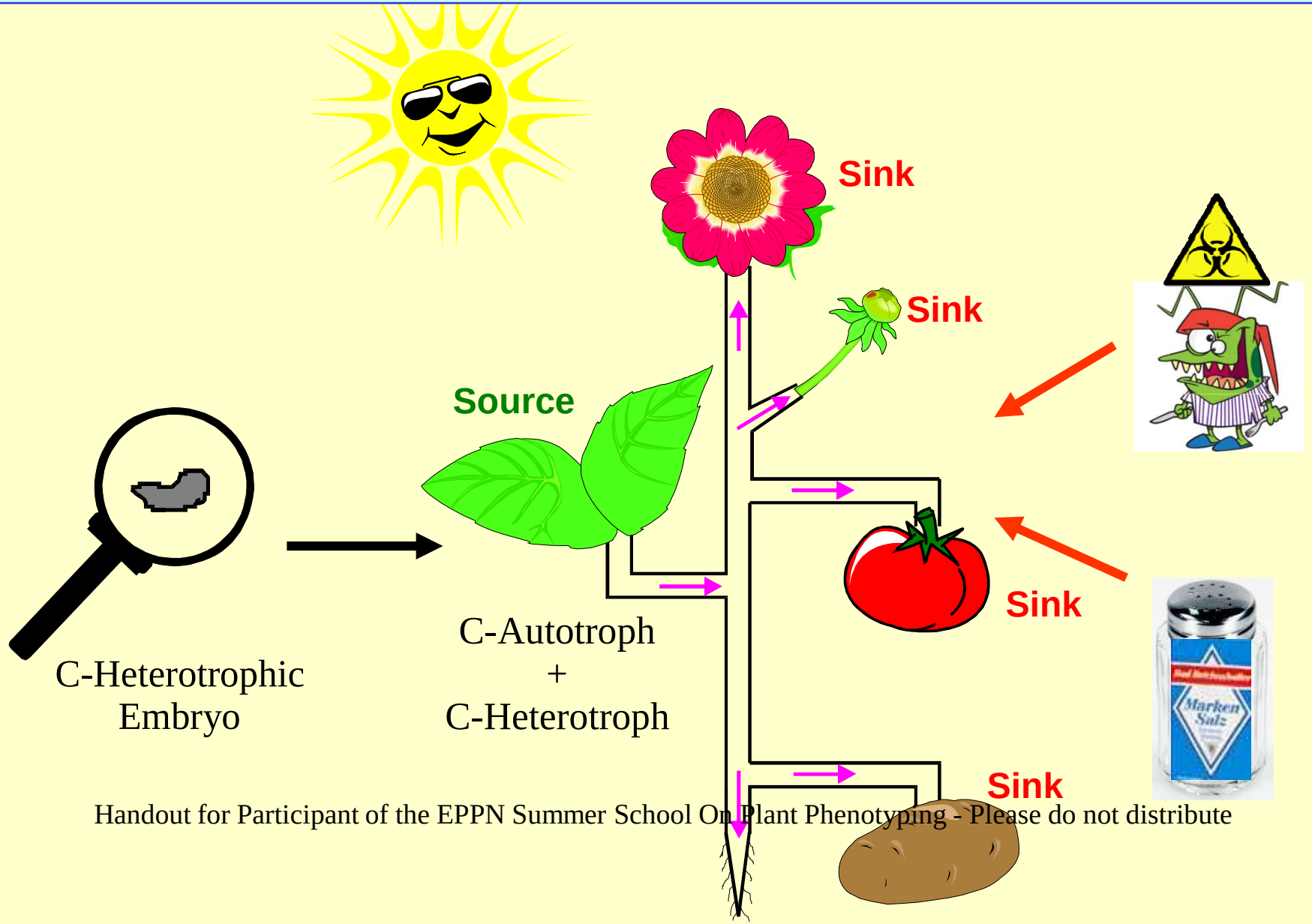
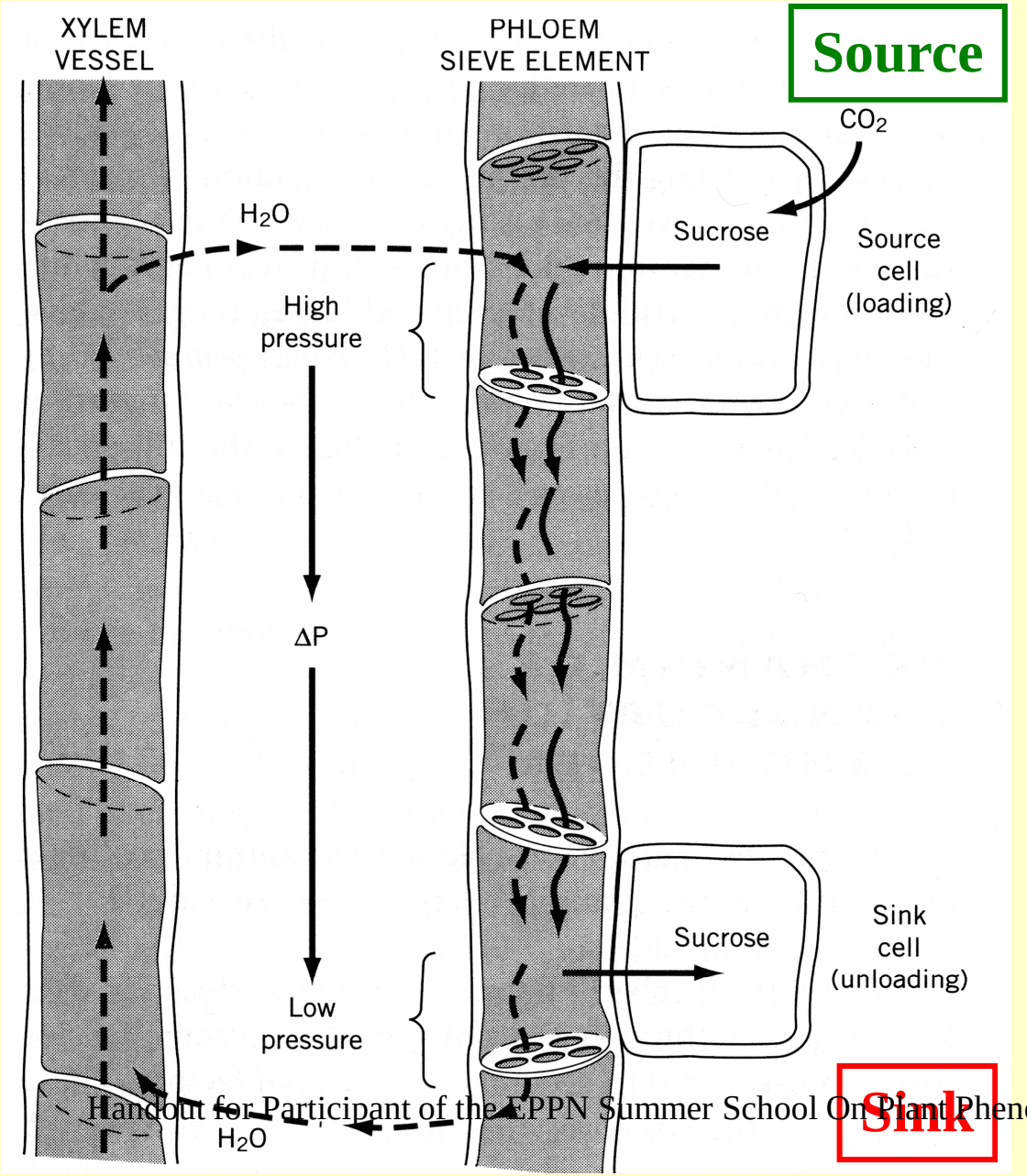
1. Introduction to carbohydrate partitioning, source-sink-relations and phloem unloading mechanisms:
   - Essential role of extracellular invertase: Pollen development
   - Relation between hormones and carbohydrates: Cytokinin
2. Co-ordinaton of source-sink-relations and biotic stress responses: Fluorescence-signatures of pathogen infection
3. Sucrose metabolism and abiotic stress: Fluorescence-signatures drought stress responses
4. Integration of fluorescence signatures with physiological phenotyping: PHENOMICS





1. Introduction to carbohydratre partitioning, source-sink-relations and phloem unloading mechanisms

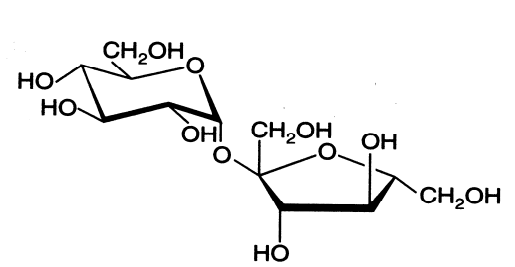




Assimilat-Transport

Pressure Flow Mechanism
according to Münch (1929)

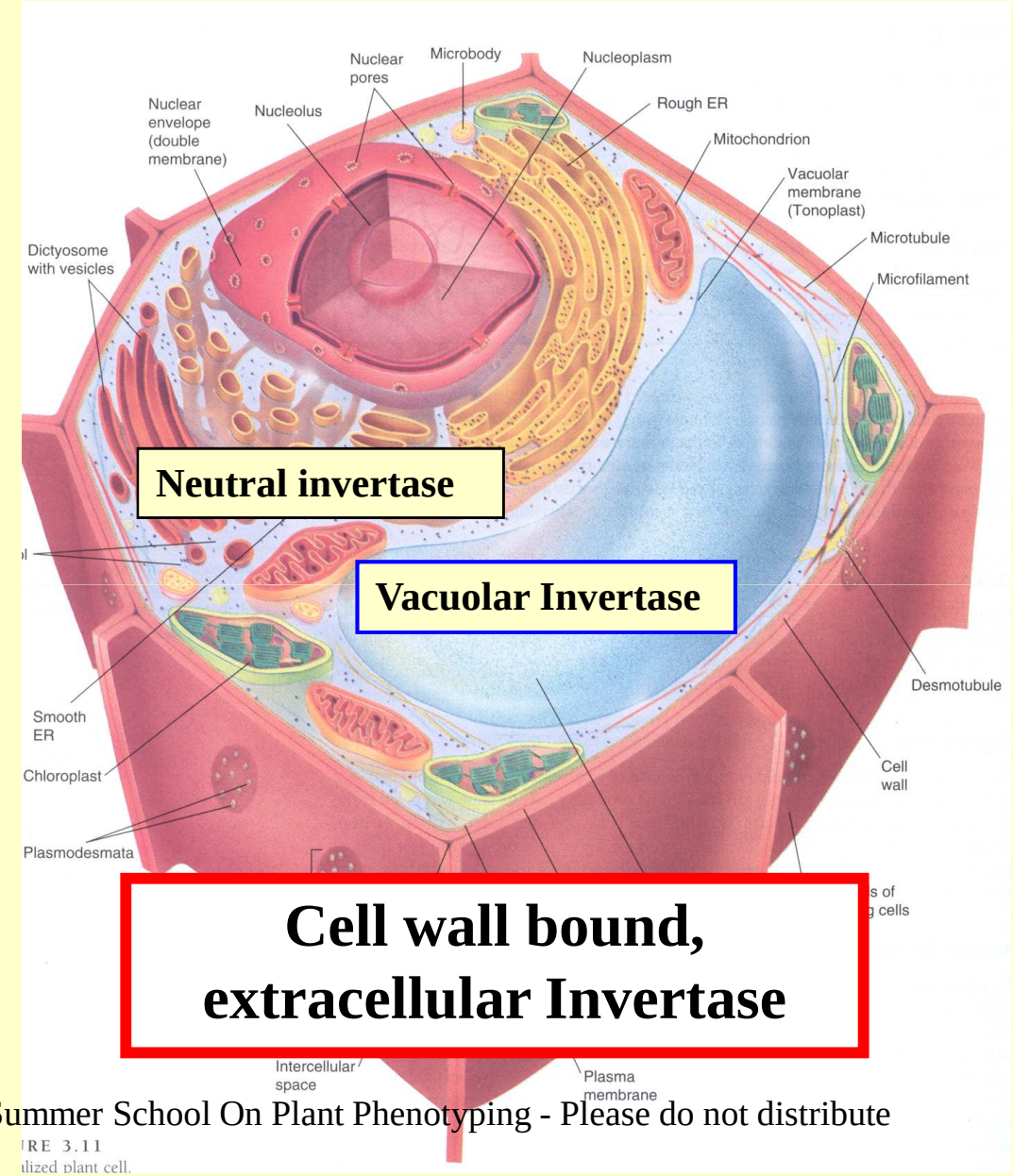
The Invertase Isoenzymes



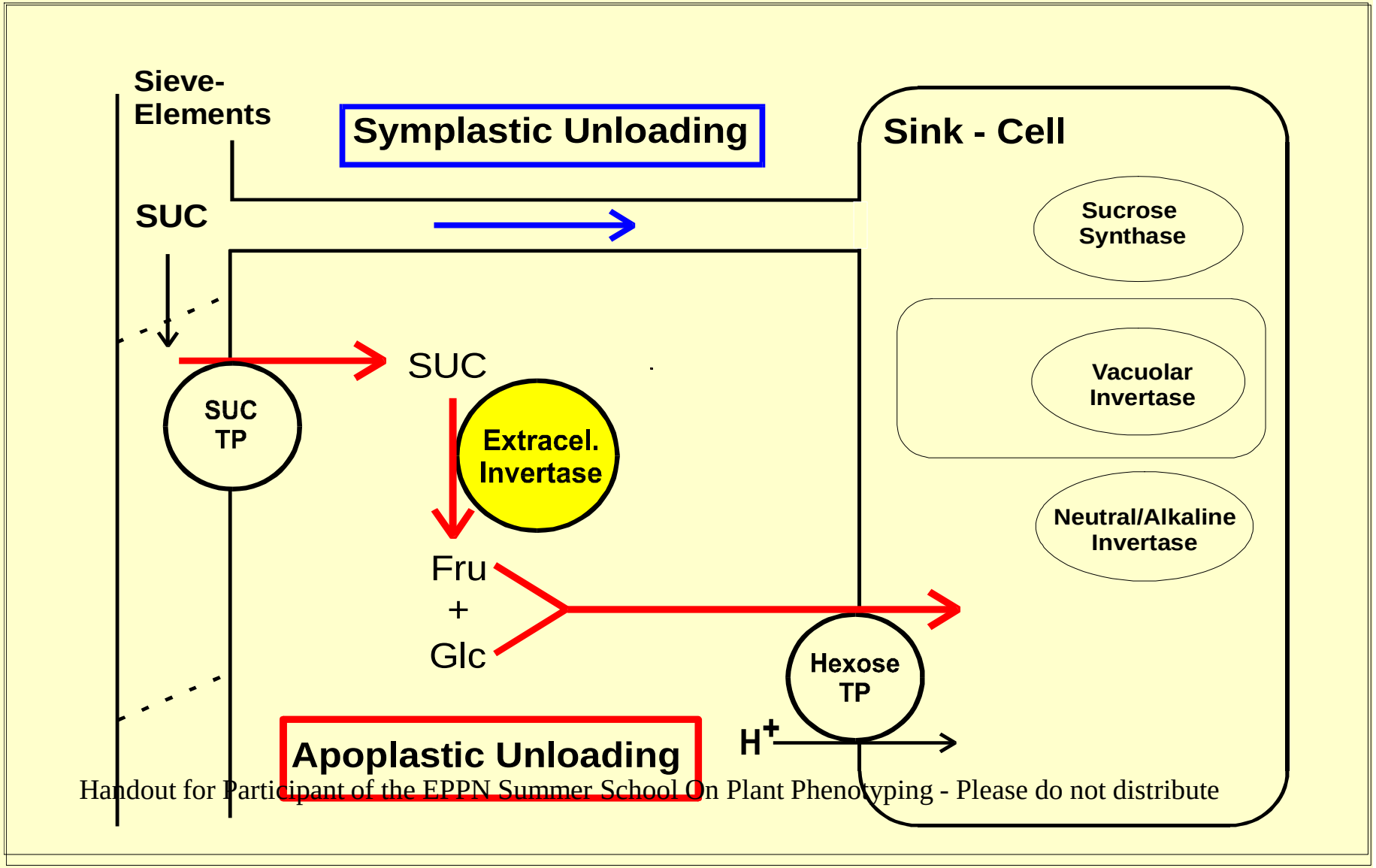
Sucrose

α-D-Glucopyranosyl-ß-D-fructofuranoside

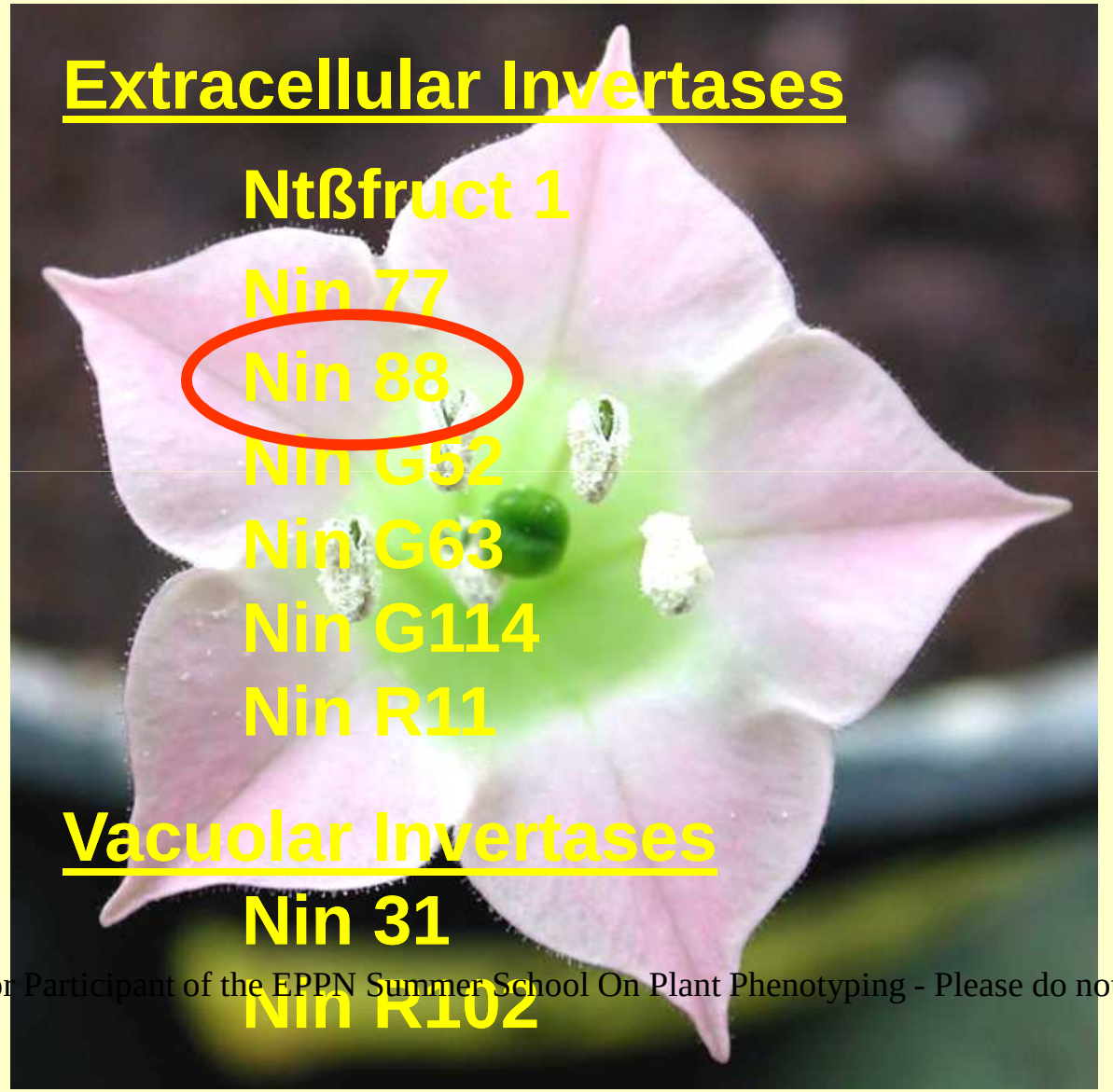
Glucose + Fructose



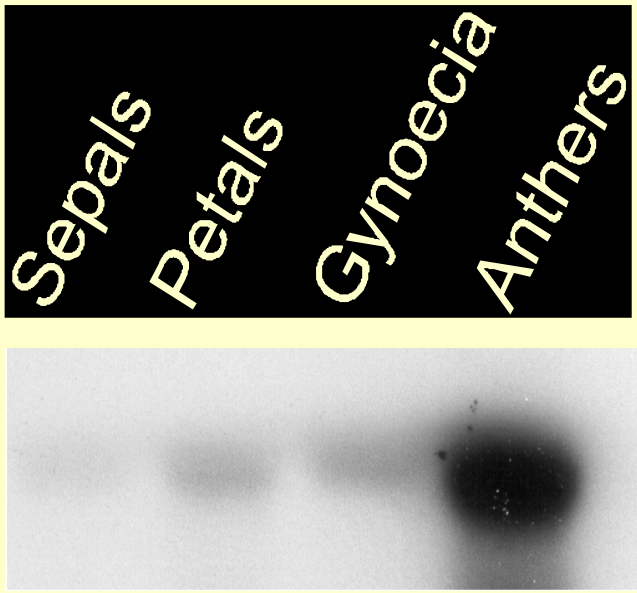
Mechanisms of Phloem Unloading



1.2 Essential role of invertase to supply carbohydrates to developping pollen

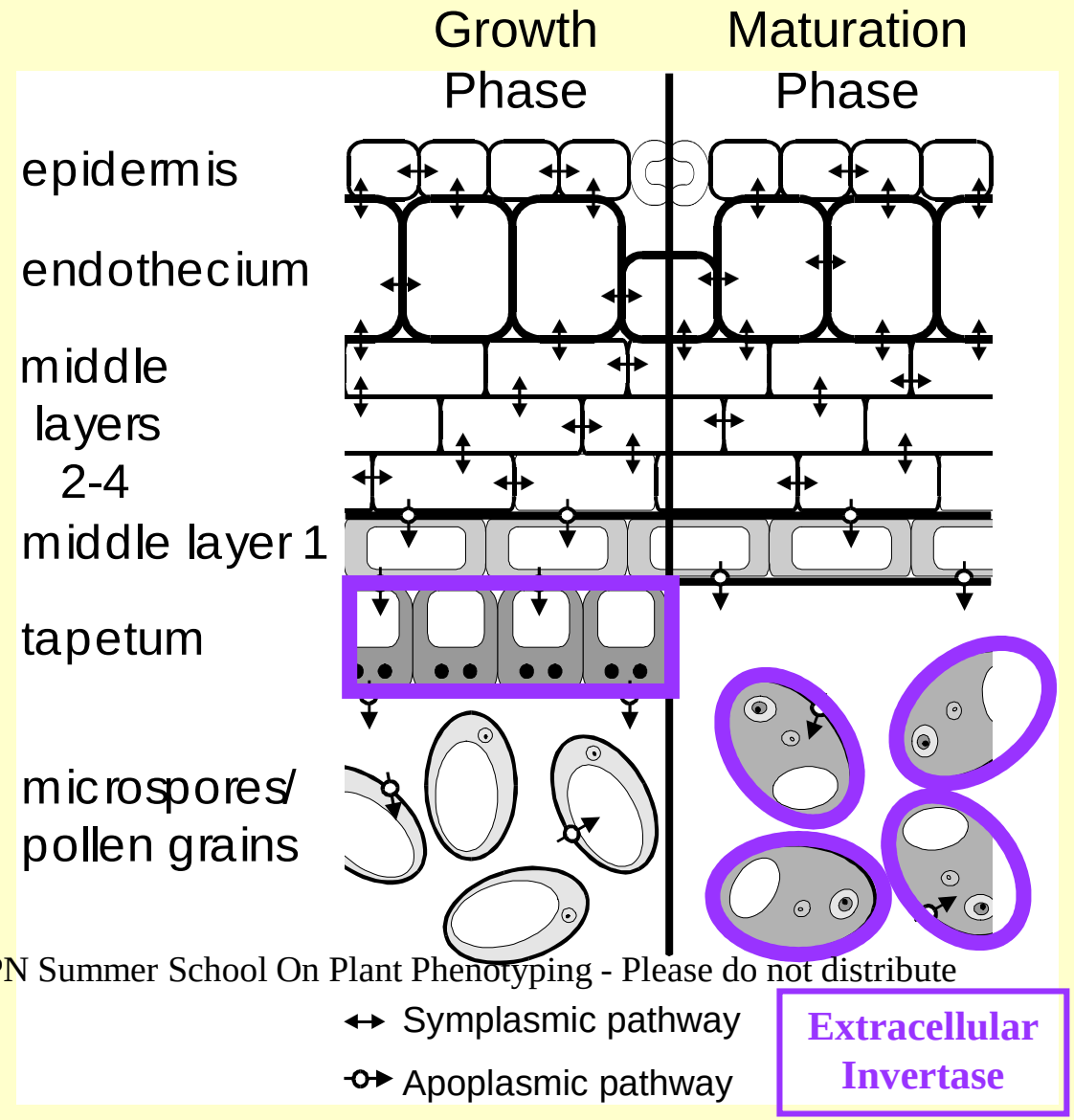


Extracellular Invertase Nin88 is specifically expressed in Anthers



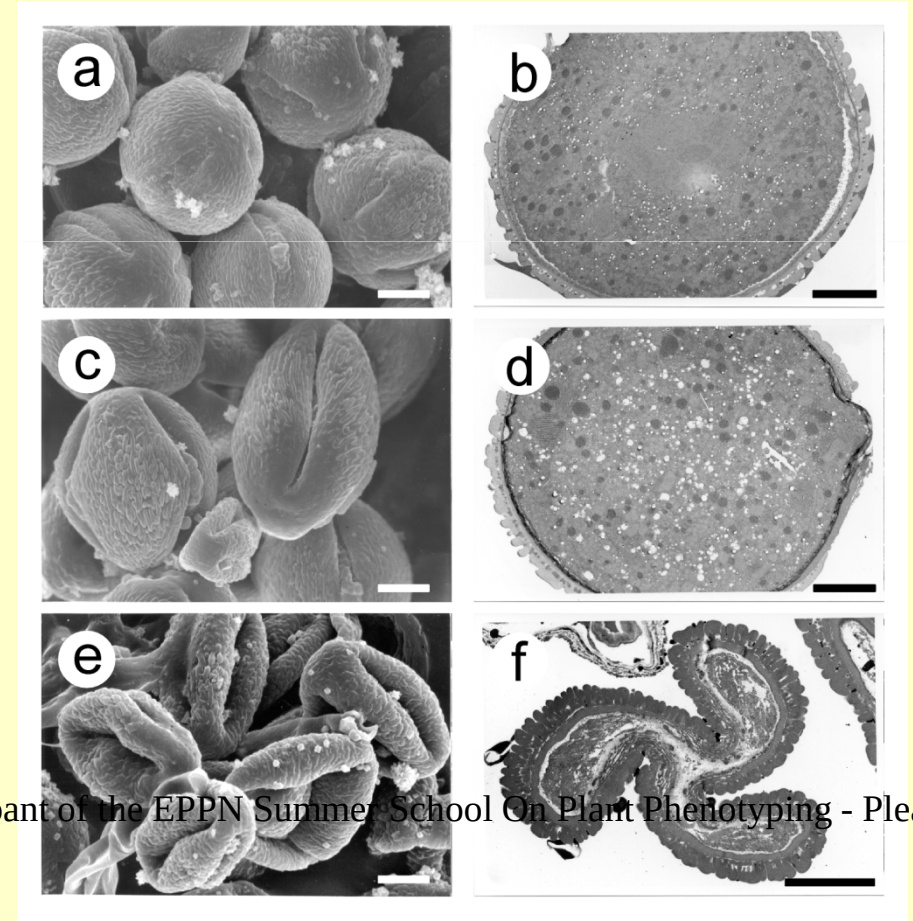
Northern Blot

Specific temporal and patial expression of extracellular invertase Nin88 during Male Gametophyte development



Antisense-Repression of Extracellular Invertase Nin88 results in an Arrest of Pollen Development

SEM (2700x) TEM (5600x)

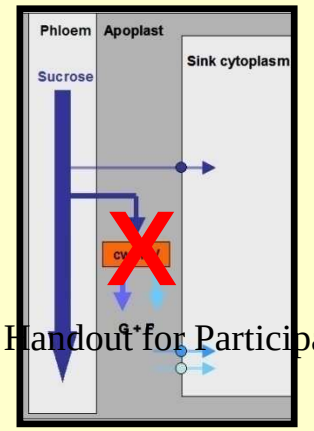


Wildtype

NT23-17

NT23-59

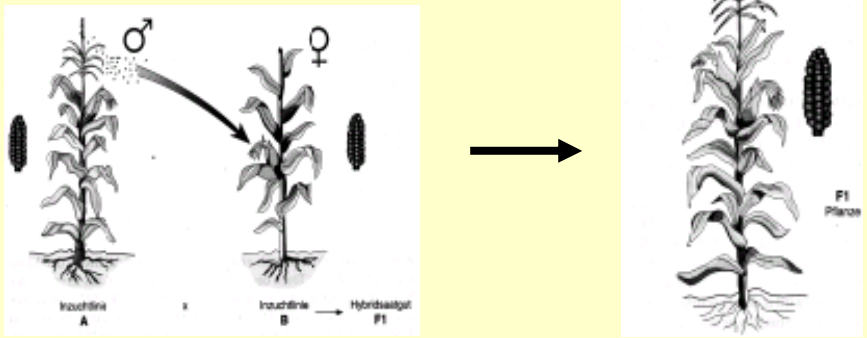
Nin88::
Nin88-Antisense



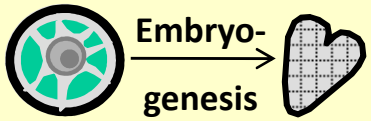
Goetz et al. (2001) PNAS

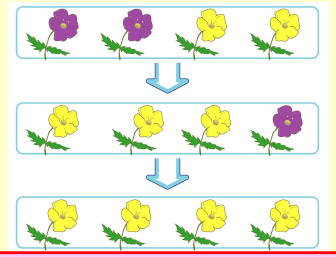
Biotechnological application of the generation of male sterile plants by metabolic engineering of the carbohydrate supply of anthers

•Hybridseed production



•Plant Breeding



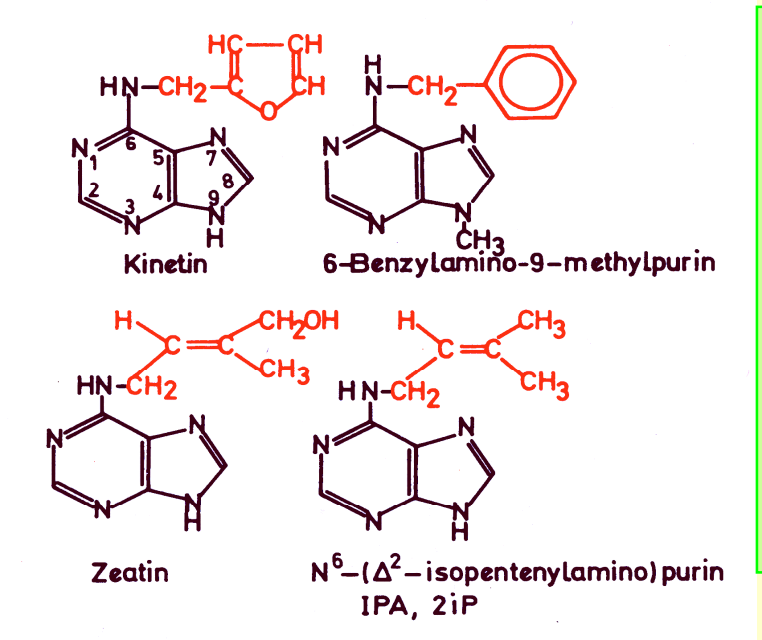


•Biological safetey procedure
avoid outcrossing of transgen

➢ **Extracellular invertase is essential for pollen development and also involved in pollen germination and pollen tube growth**

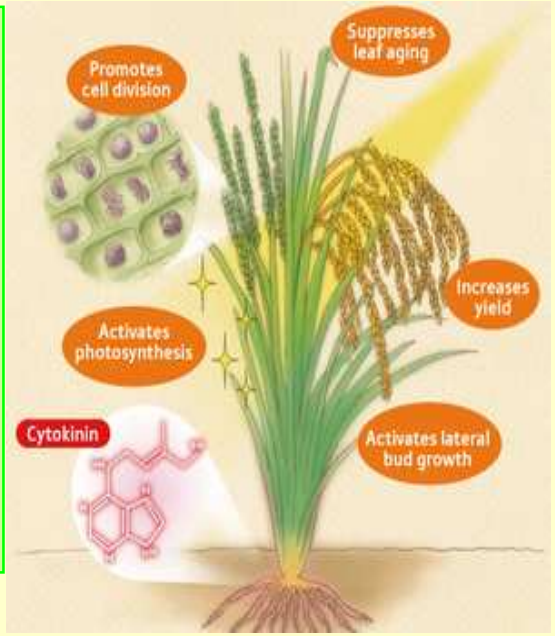
1.2 Relation between primary metabolism and phytohormone action

Structure and Function of Cytokinins



Effects:

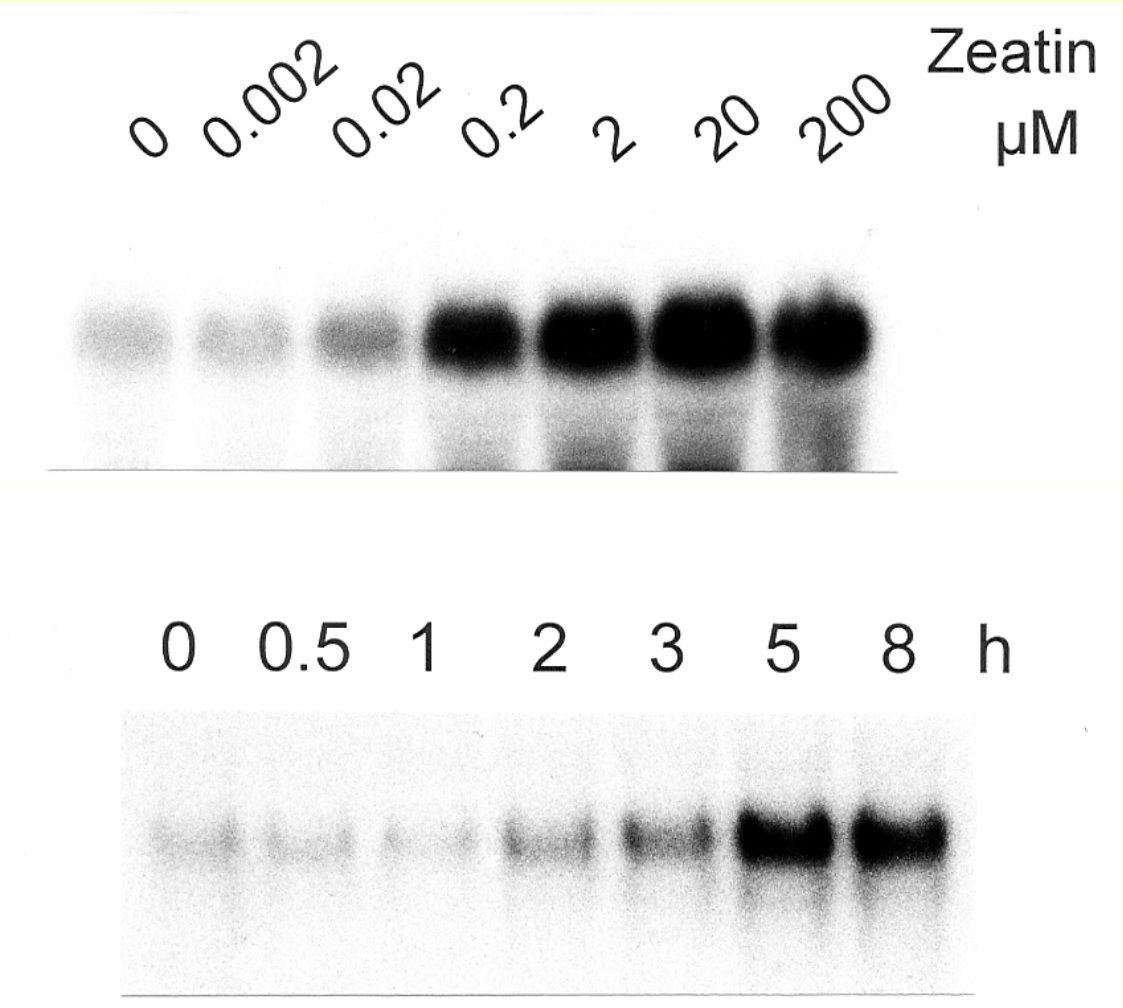
- Cell division
- Morphogenesis
- Growth of lateral buds
- Leaf expansion
- Delay of senescence



Produced by :

- Plants
- Microbial Pathogens
- Insects

Induction of Extracellular Invertases by Cytokinin



Induction of the mRNA for extracellular invertase Cin1 in a photoautotrophic culture of *Chenopodium rubrum*

Delay of senescence and nutrient attraction by cytokinins

K. Mothes, Halle

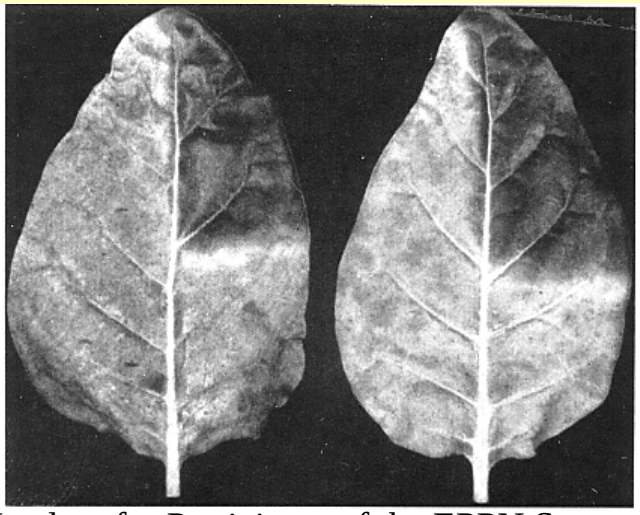
DIE NATURWISSENSCHAFTEN

47. Jahrgang Heft 15 (Erstes Augustheft) 1960

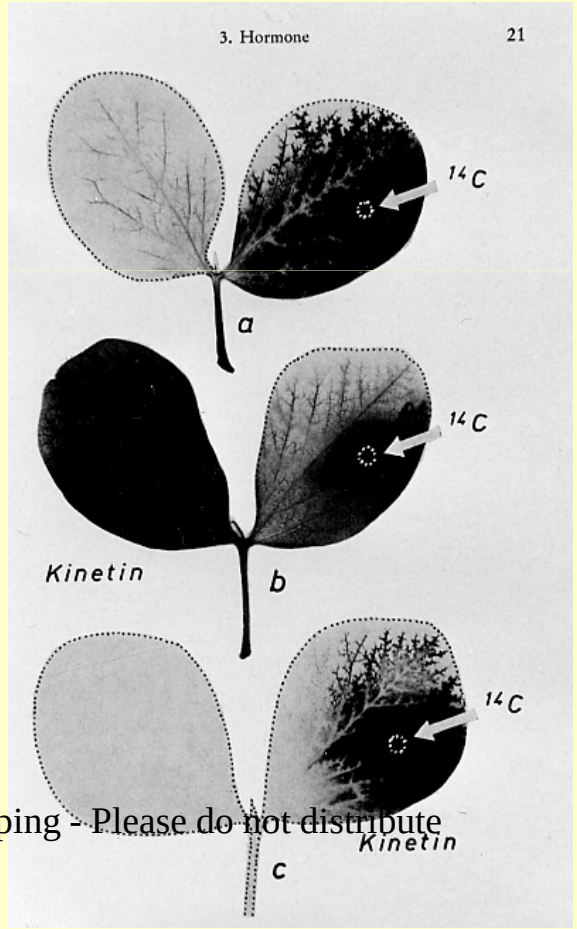
Über das Altern der Blätter und die Möglichkeit ihrer Wiederverjüngung

Von K. MOTHES, Halle/Saale

Dem Andenken an WILHELM RUHLAND († 5. 1. 1960) gewidmet.

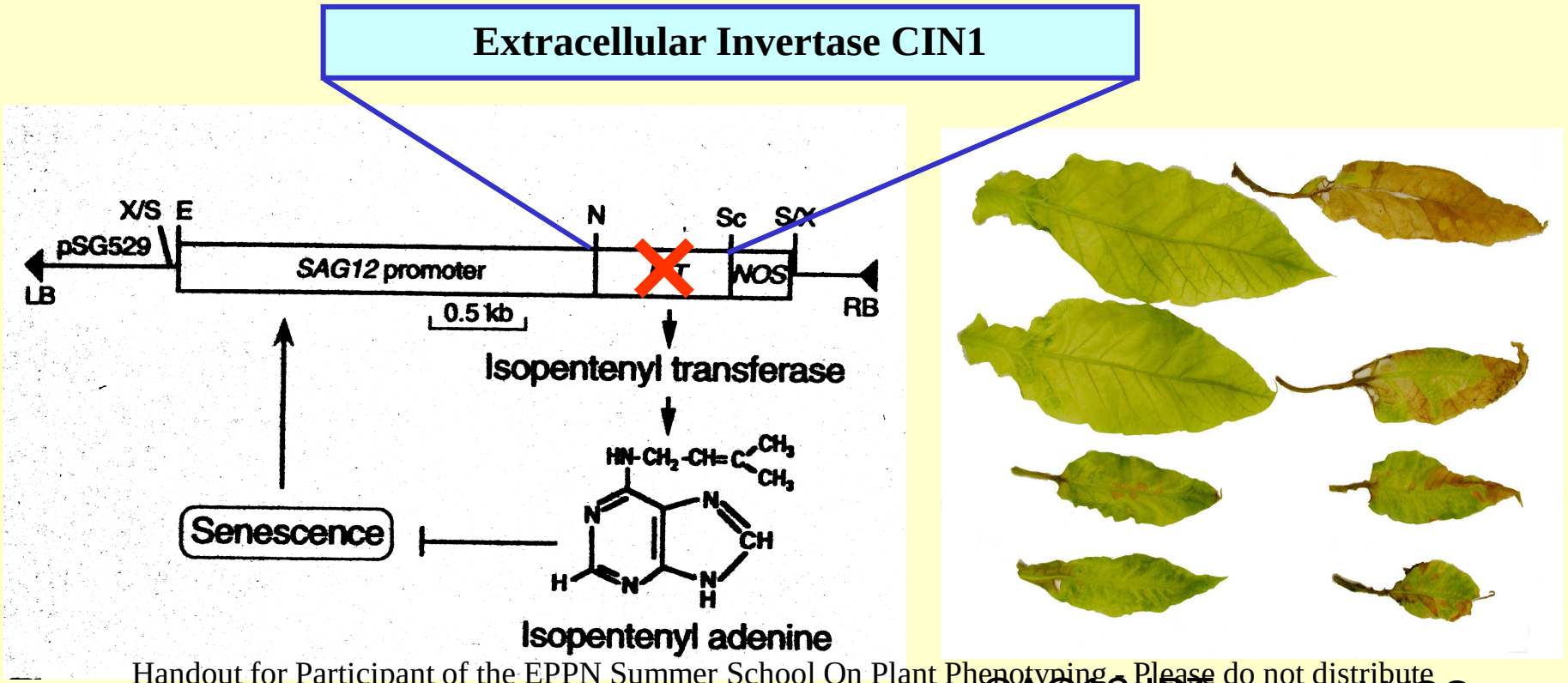


einmal mit Kinetinlösung besprüht (30 mg/Liter). Der Kinetin-Bezirk ist nach 10 Tagen noch grün, während die übrigen Spreitenteile schon absterben



Inhibition of Leaf Senescence by Autoregulated Production of Cytokinins

S. Gang and R. M. Amasino
Science ♦ Vol. 270 ♦ 1995 ♦ p. 1986 - 1989



Senescence induced Extracellular Invertase CIN1 results in Delay of Senescence

Cytokinin ➔ Extracellular Invertase ➔ Delay of Senescence

SAG12: Cin1

W 38

Senescence induced Extracellular Invertase CIN1 results in Delay of Senescence

Cytokinin ➔ Extracellular Invertase ➔ Delay of Senescence

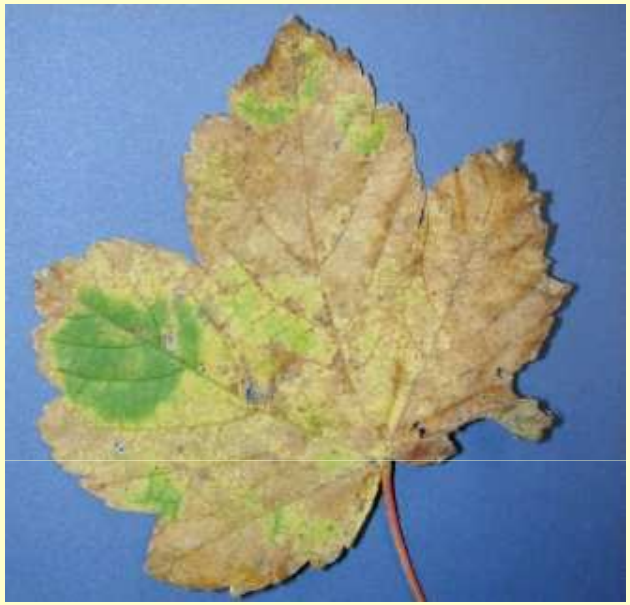
SAG12: Cin1

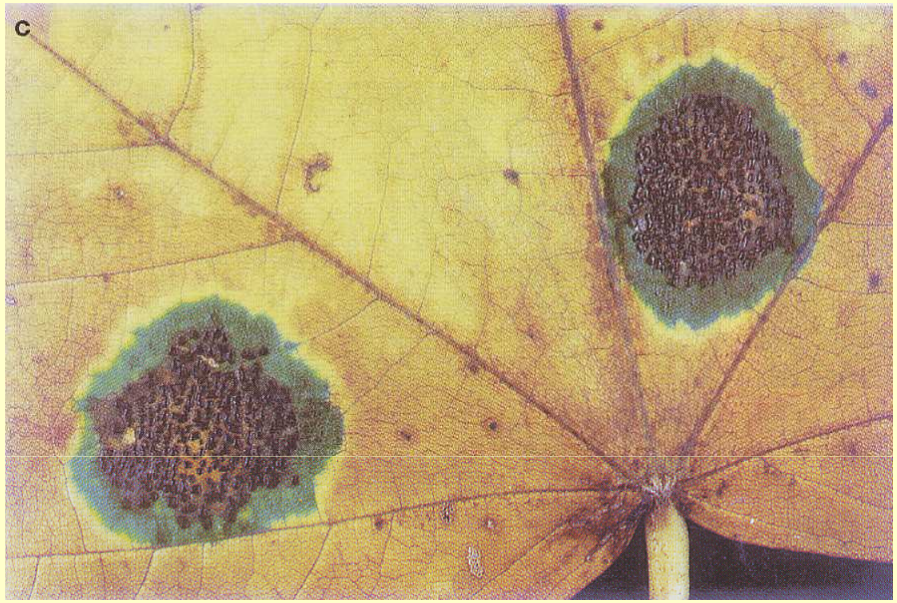
W 38

Balibrea et al.. (2004) Plant Cell

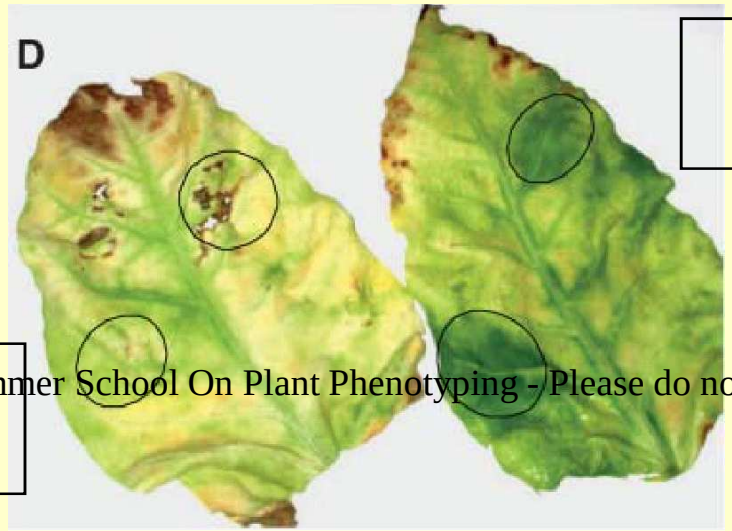
Green Islands in Autumn Leaves

Delay of senescence by pathogen derived cytokinins





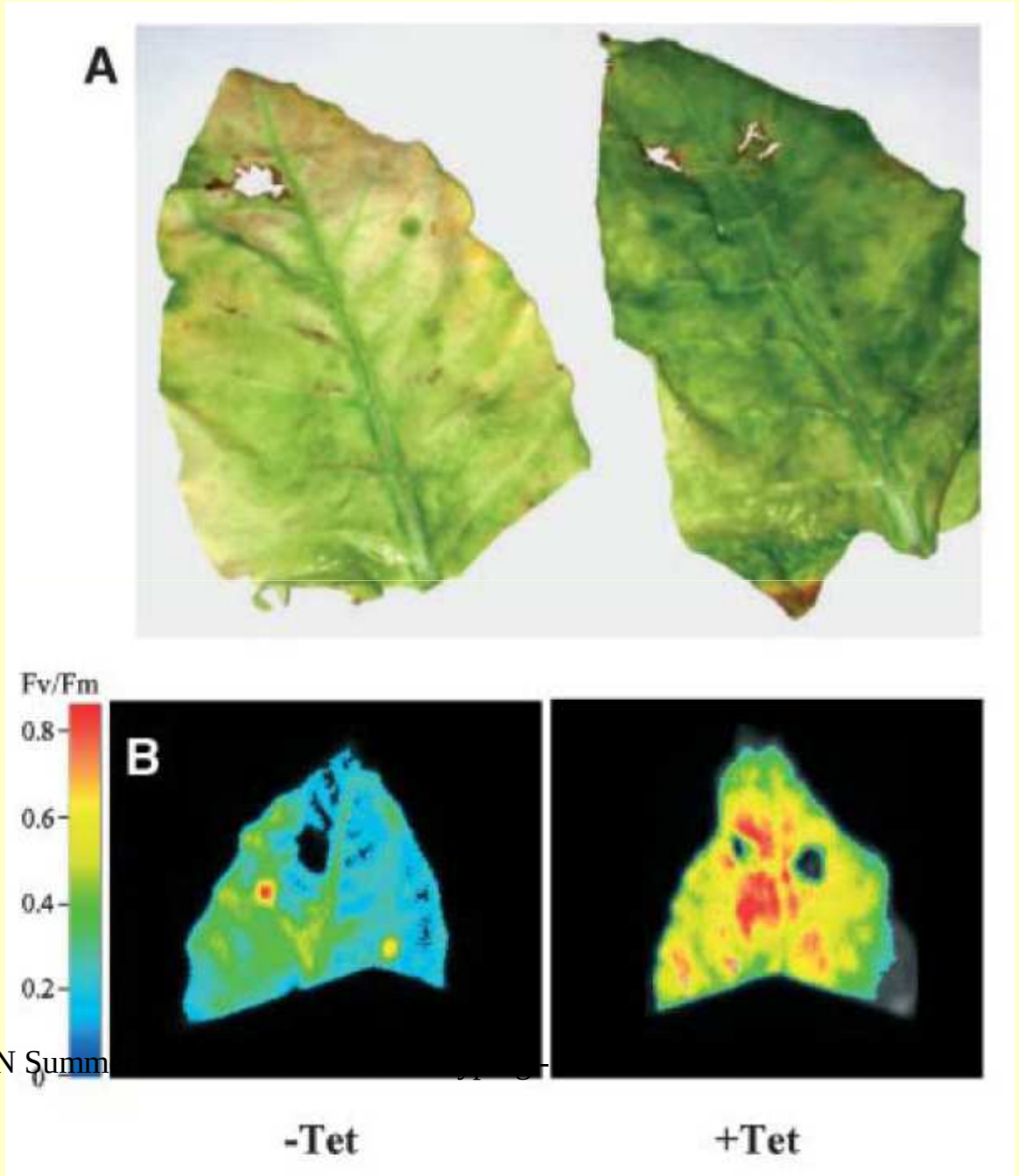
Ectopic induction of an extracellular invertase in tobacco plants: *Tet::Cin1*



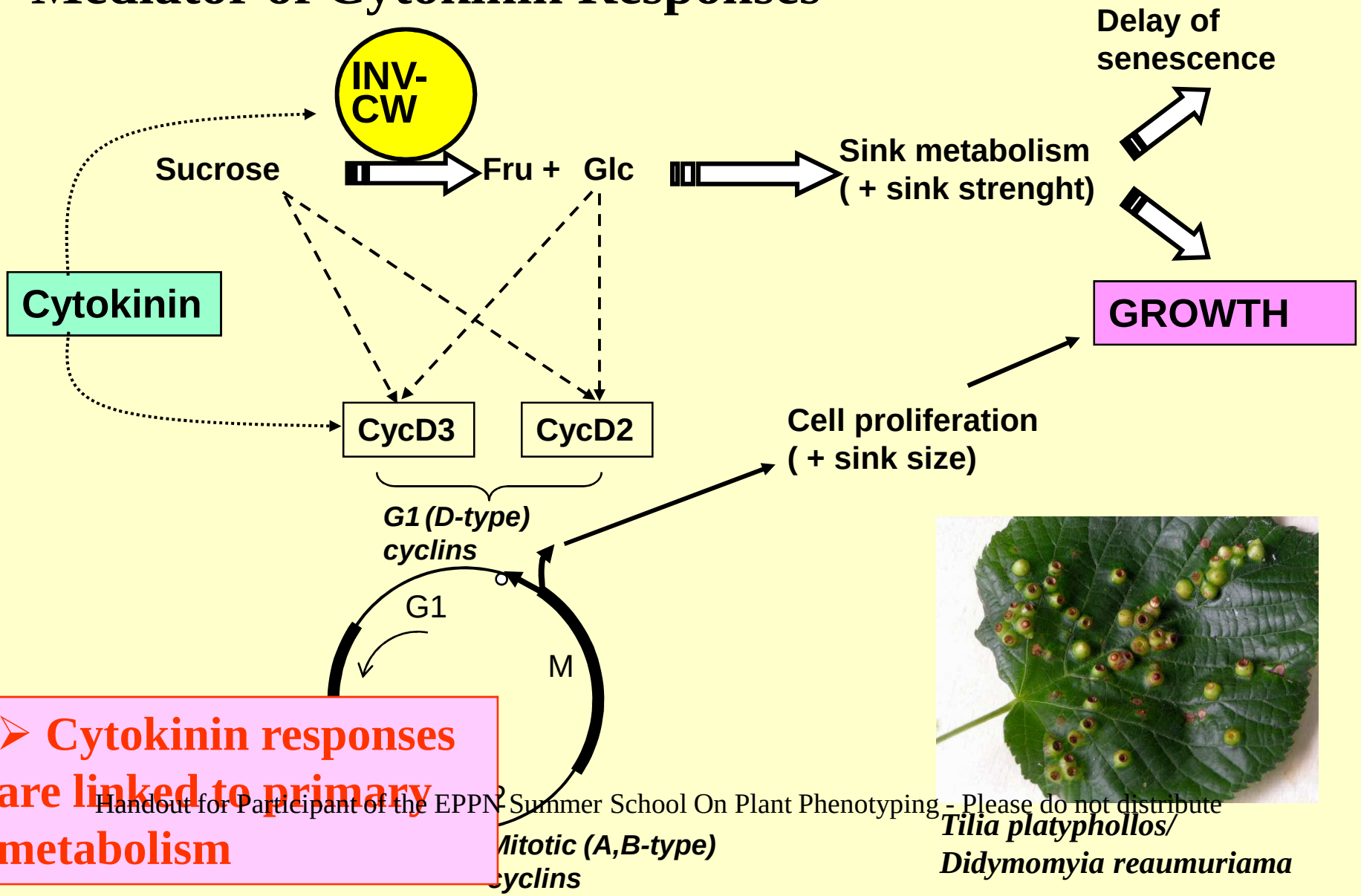
Tetracyclin treatment

Mock treatment

Ectopic induction of an extracellular invertase in tobacco plants: *Tet::Cin1*



Extracellular Invertase
- Mediator of Cytokinin Responses-

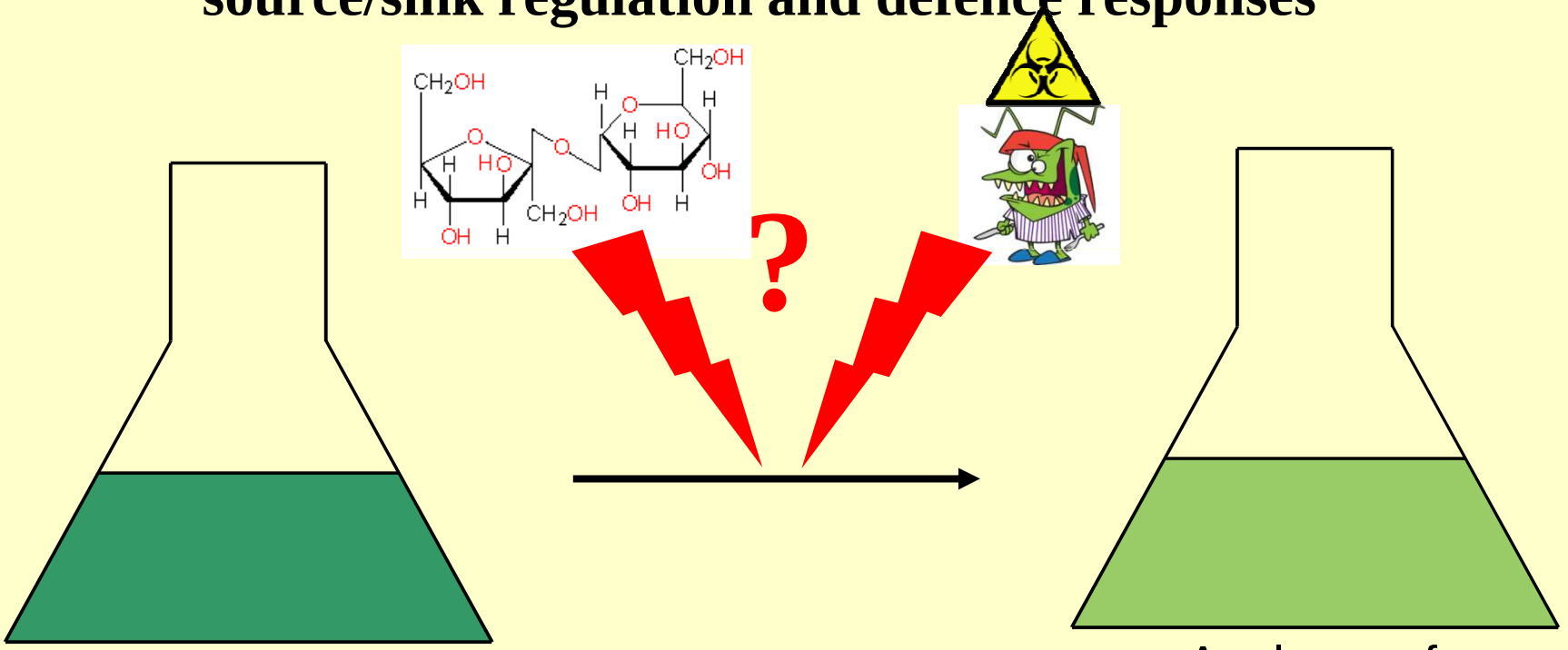


➢ **Cytokinin responses are linked to primary metabolism**

Tilia platyphollos/
Didymomyia reaumuriama

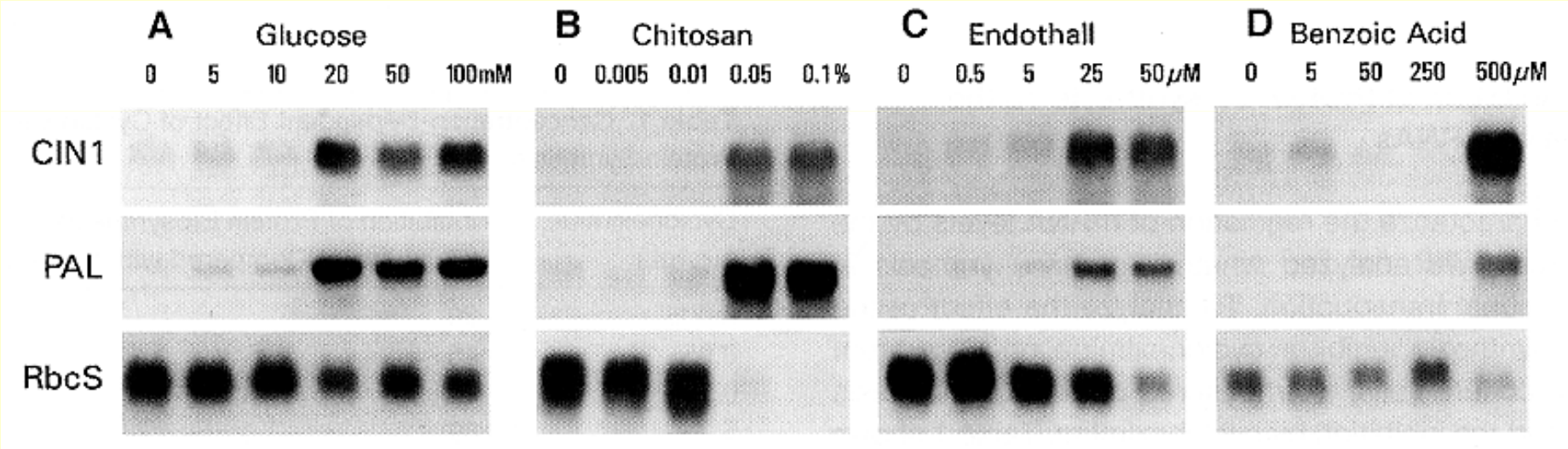
2. Co-ordinaton of source-sink-relations & biotic stress responses: Fluorescence-signatures of pathogen infection

Photoautotrophic cultures: an experimental system to study source/sink regulation and defence responses



Co-ordinated Regulation of Source-/Sink-Metabolism and Defence Responses by Glucose und Stress related Stimuli in photoautotrophic Suspension Cultures

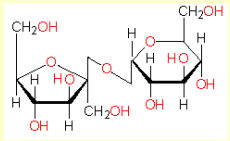
Dose response



Northern Blot

Ehness et al. (1997) Plant Cell 9: 1825

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



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

Infection of Tomato with the necrotrophic fungus *Botrytis cinerea*

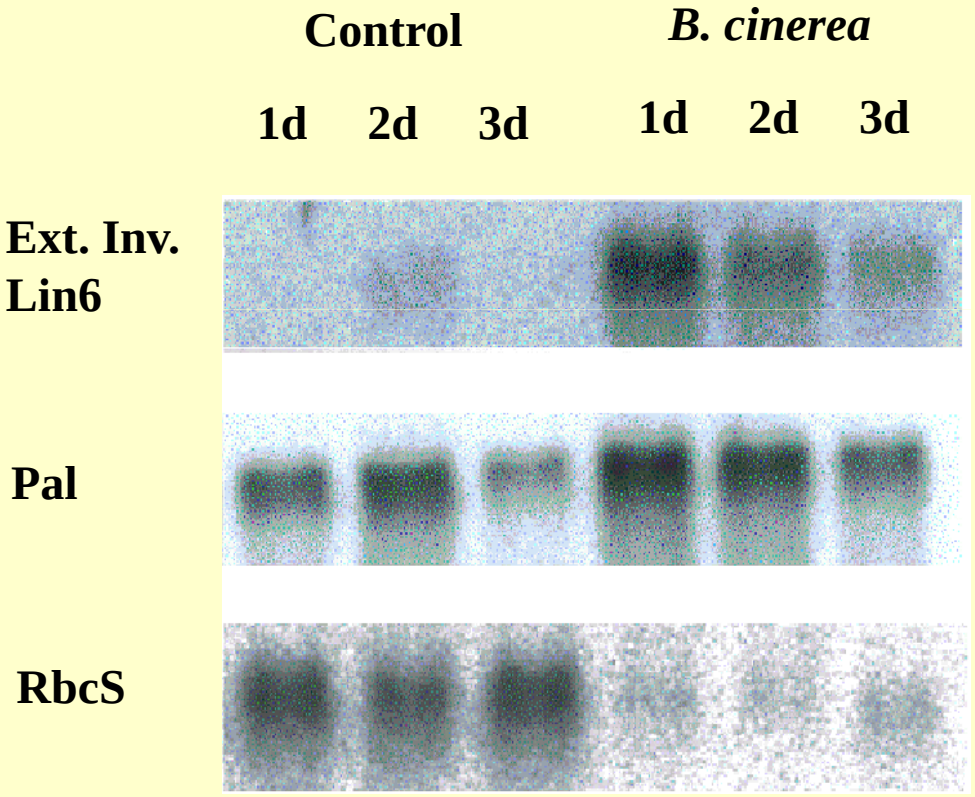
2d

3d

6d

***Solanum esculentum* cv. Mikrotom**

Co-ordinated Regulation of Source-/Sink-Metabolism and Defence Responses in tomato leaves infected by *Botrytis cinerea*



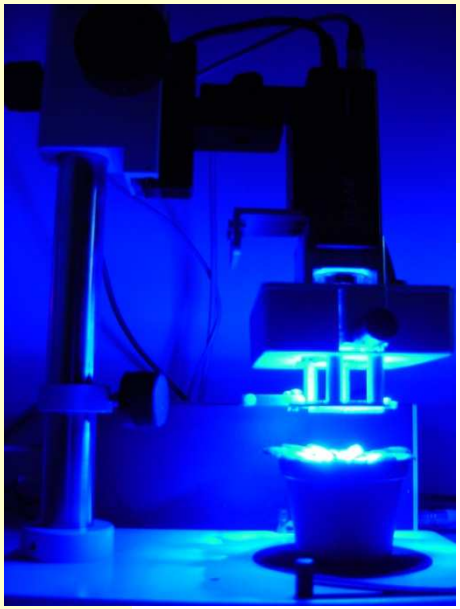
Northern Blot

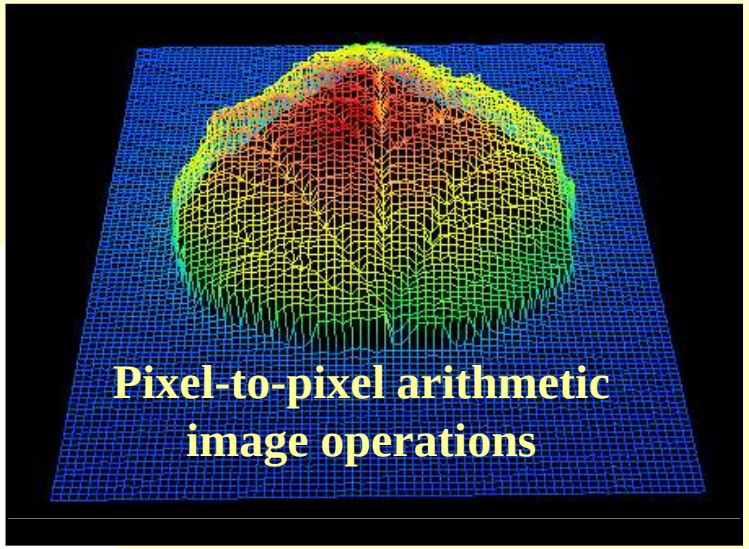
The higher invertase activity results in an increase of the hexose/sucrose ratio

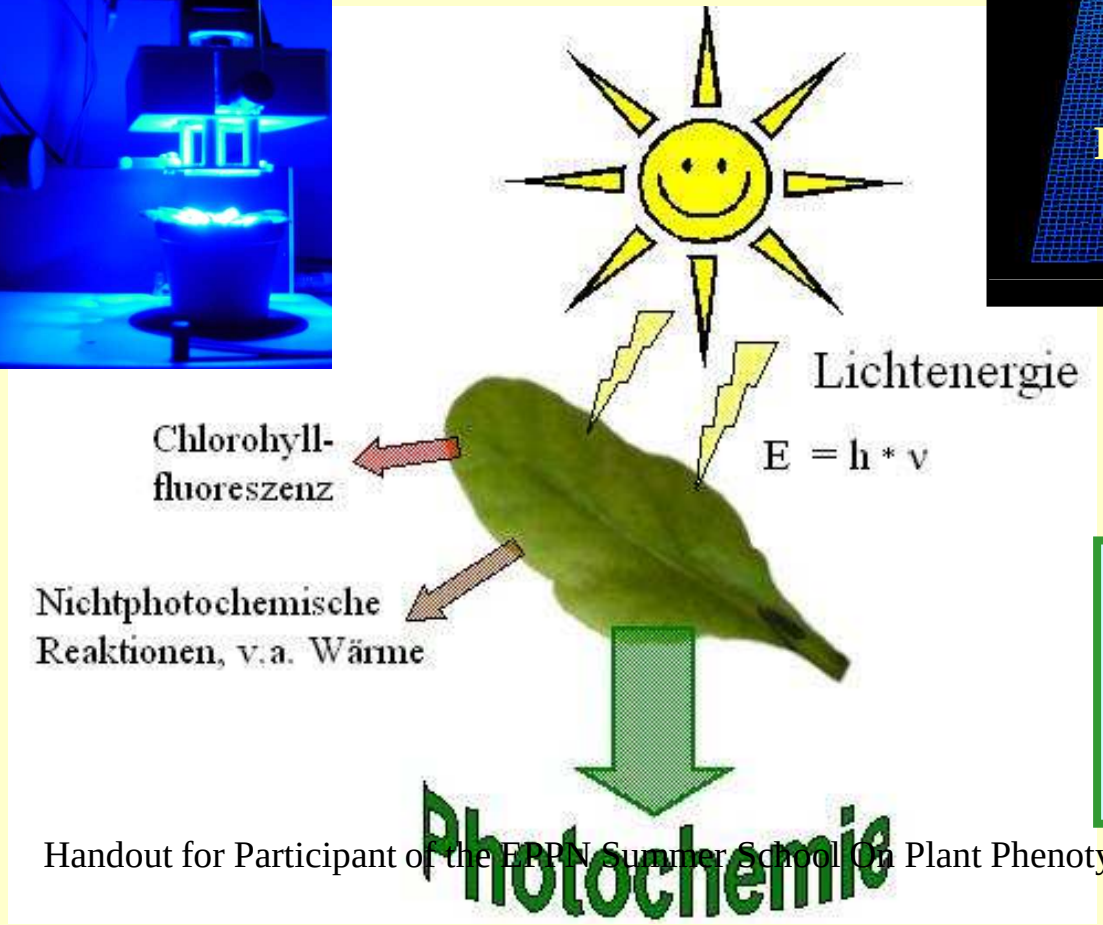
Probe	Hexose / Saccharose
malt 1d	5
malt 2d	8
malt 3d	5
B. cinerea 1d	28
B. cinerea 2d	27
B. cinerea 3d	30

Berger et al. (2004) Physiol. Plant. 122: 419-428

Chlorophyll Fluorescence Imaging







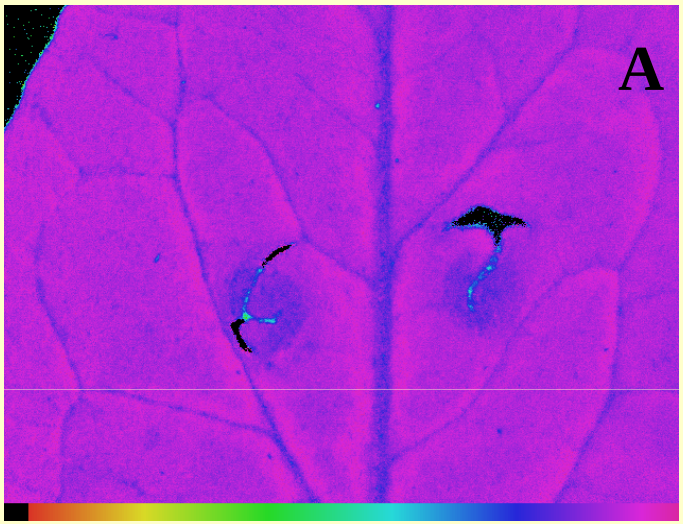
Advantages:

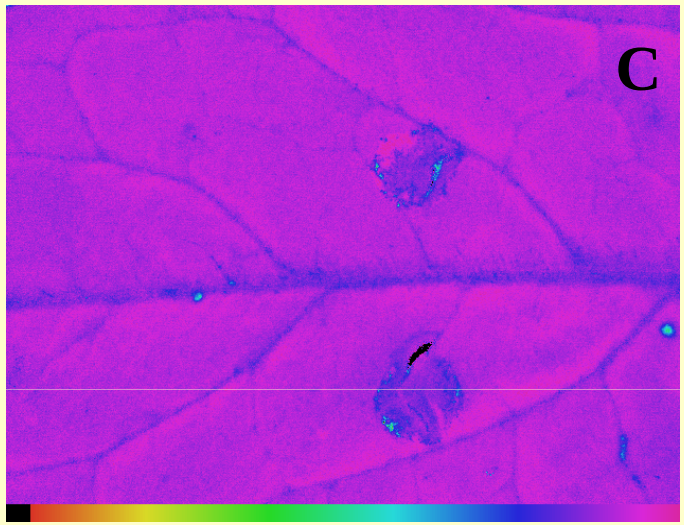
- Non invasive
- Very high sensitivity
- Spatial resolution

Visualization of spatial effects on photosynthesis by Chlorophyll Fluorescence Imaging

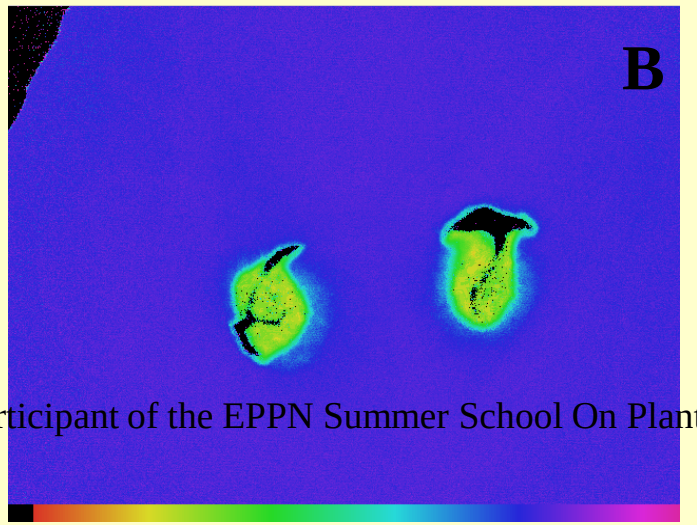
B. cinerea, 24 p.i. | Control

PAR-(Photosynthetic Active Radiation) absorptivity





Fv/Fm (Optimal quantum yield)

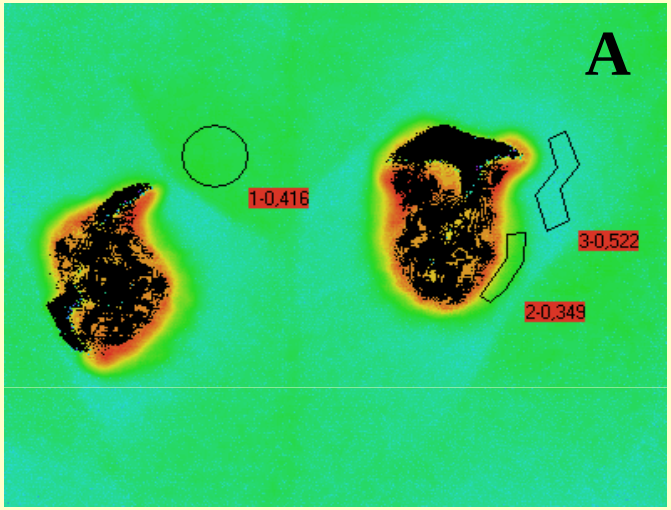


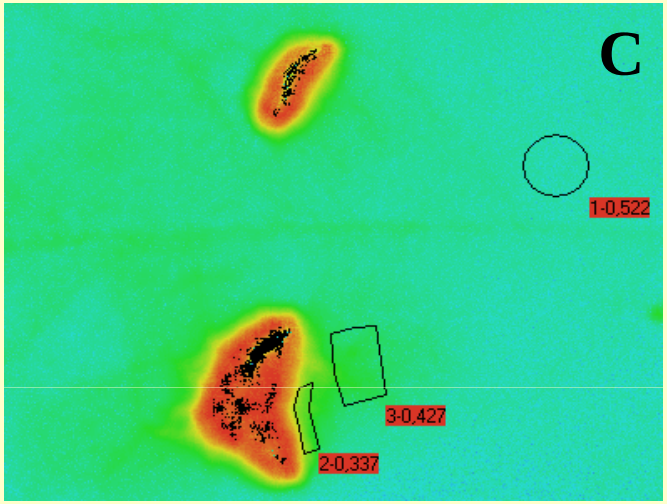
Dark adapted

Localized changes in chlorophyll fluorescence parameters in response to *B. cinerea* infection

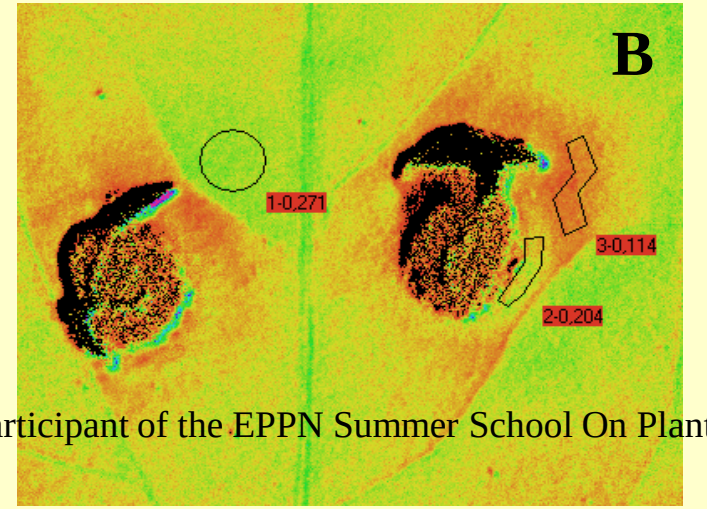
B. cinerea, 24h p.i. | Control

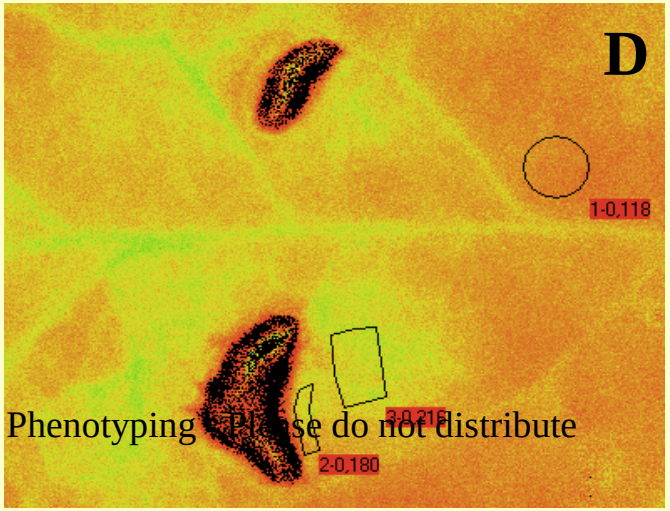
ΔF/Fm´
(Effective PSII
Quantum yield)





NPQ
(Nonphoto-
chemical
Quenching)

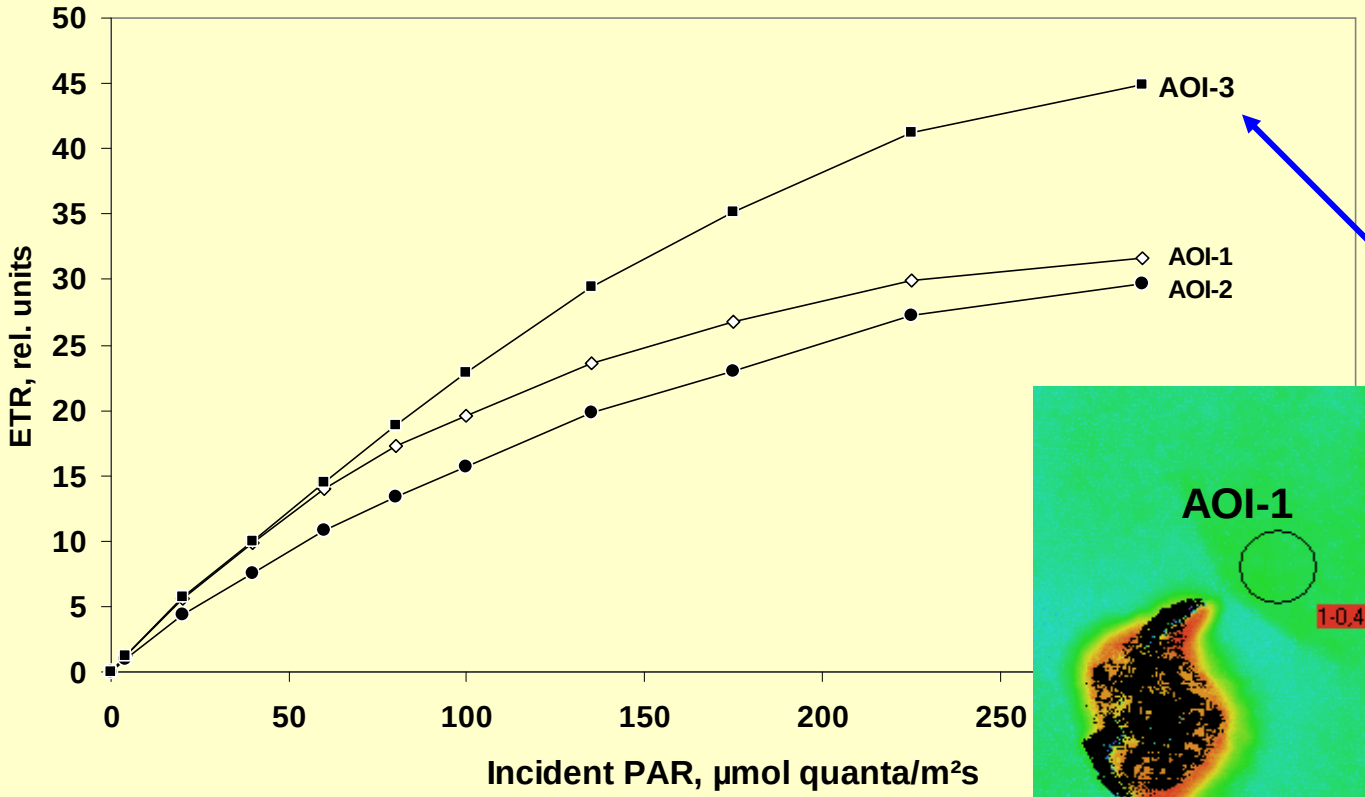


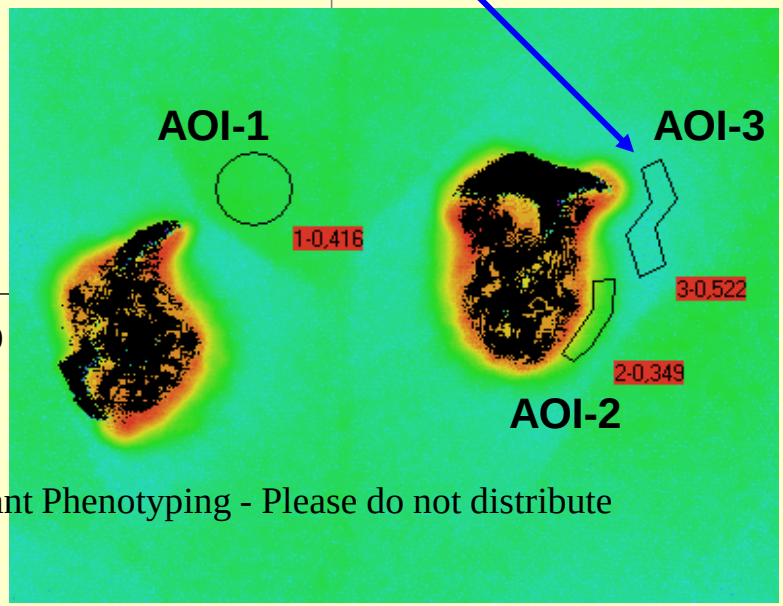


After preilumination

Localized stimulation of photosynthetic electron transport around infection sites

Light response curve

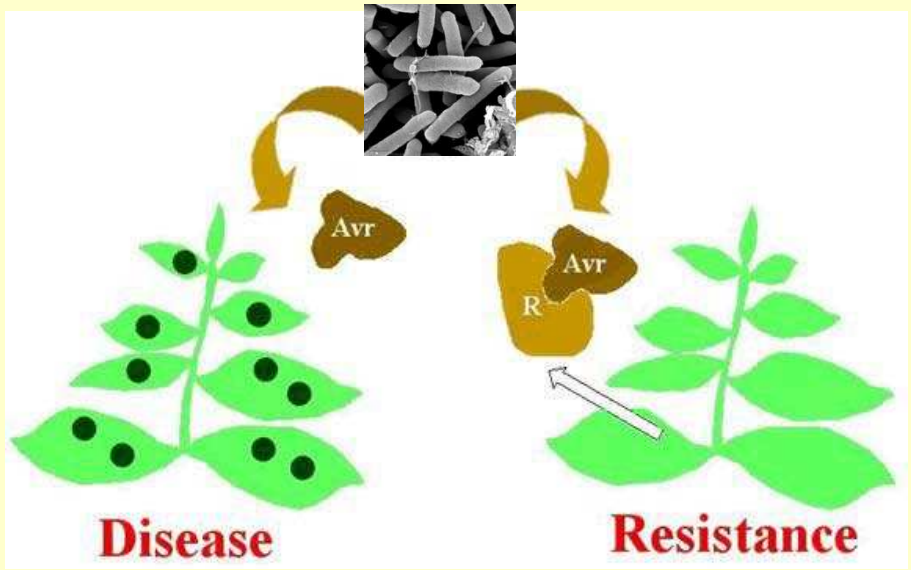


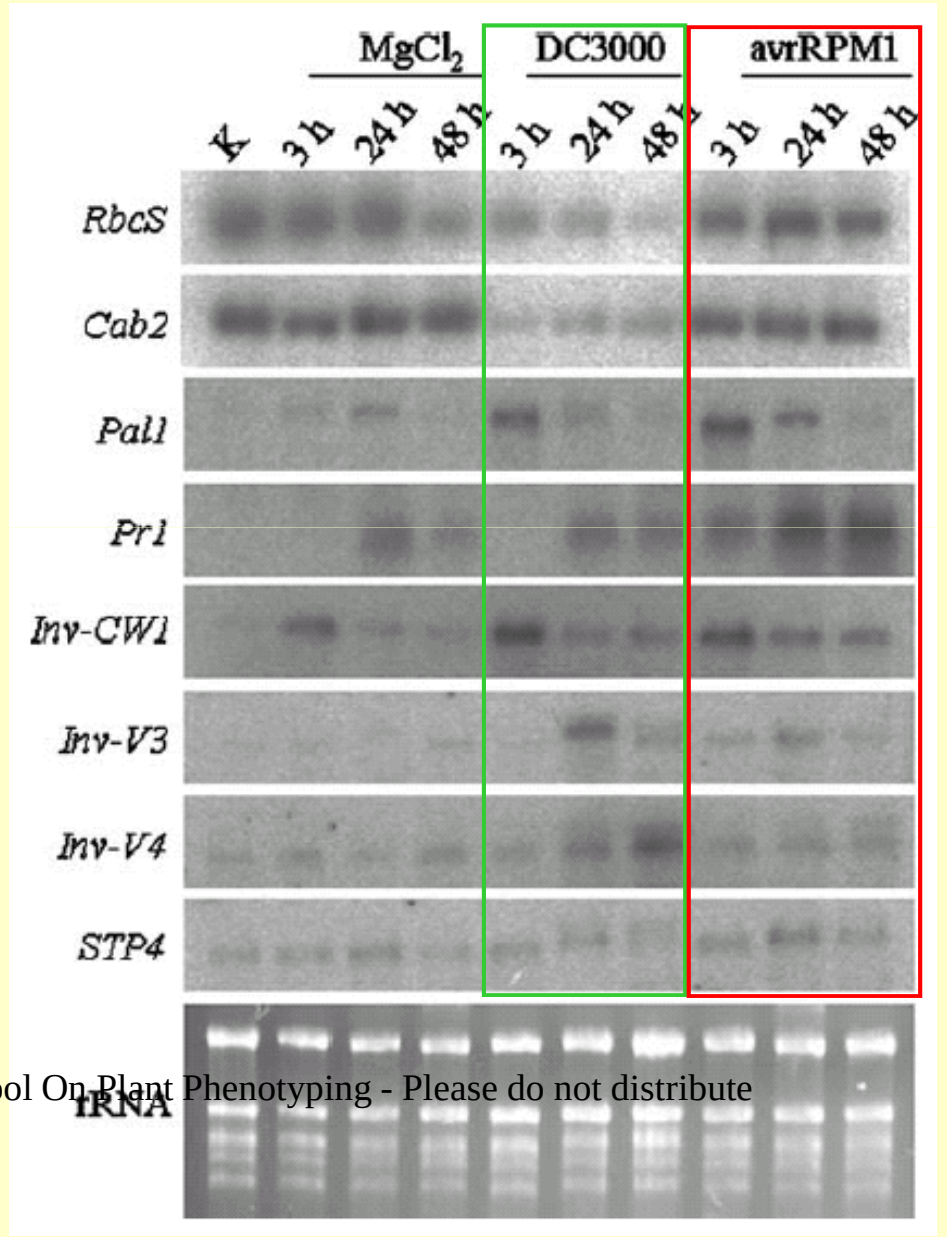


Infection of *Arabidopsis thaliana* with the hemibiotrophic bacterium *Pseudomonas syringae pv. tomato (PST)*

Category (score)	0	0.5	1	2	3	4	5
Symptoms	No symptoms	≤ 75 % Chlorosis	> 75 % Chlorosis	≤ 10 % Necrosis	11-50 % Necrosis	51-75 % Necrosis	76-100 % Necrosis
Exemplary symptoms *A. thaliana*							

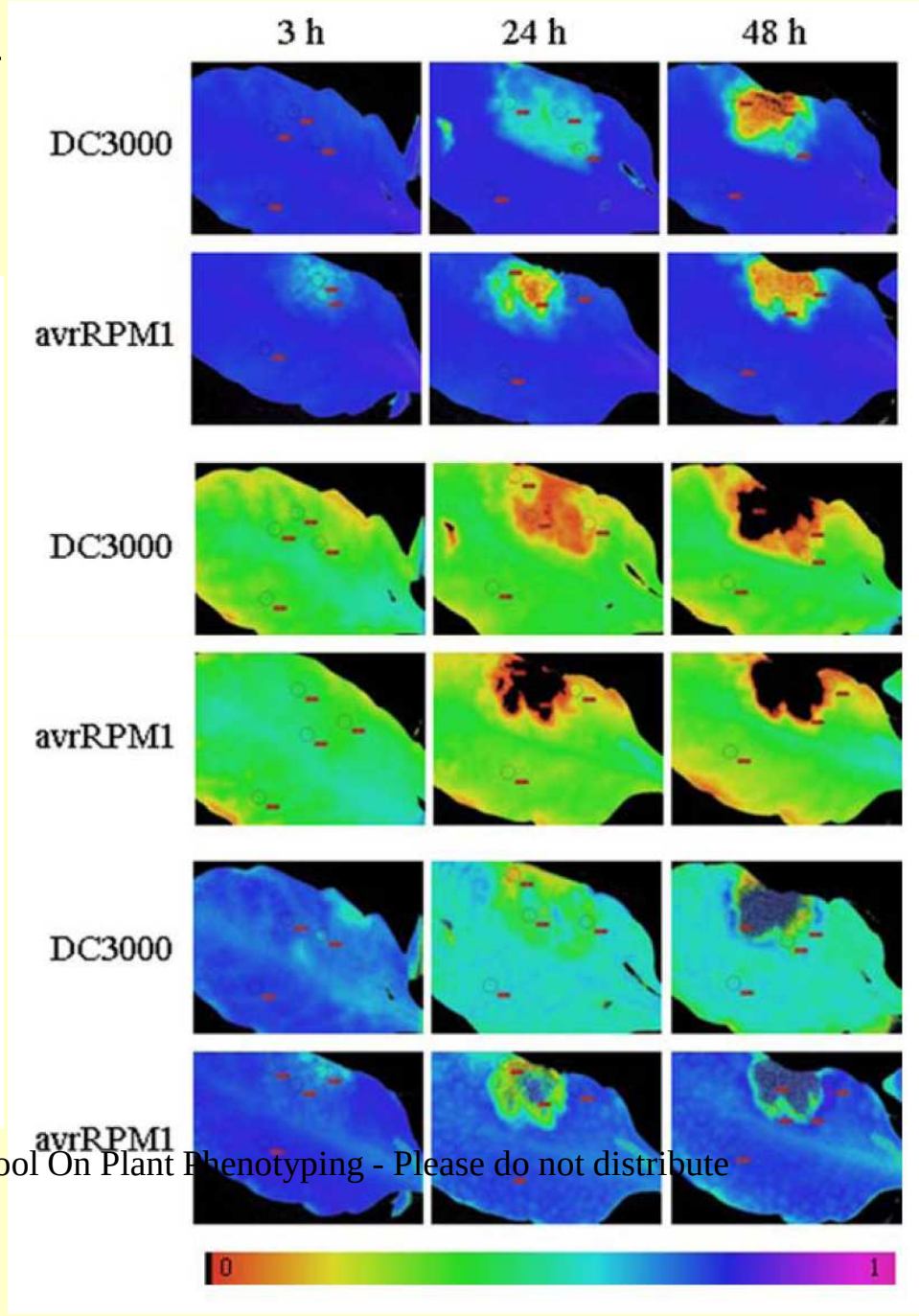
Differential effect of virulent and avirulent strains of *PST* on photosynthetic gene expression





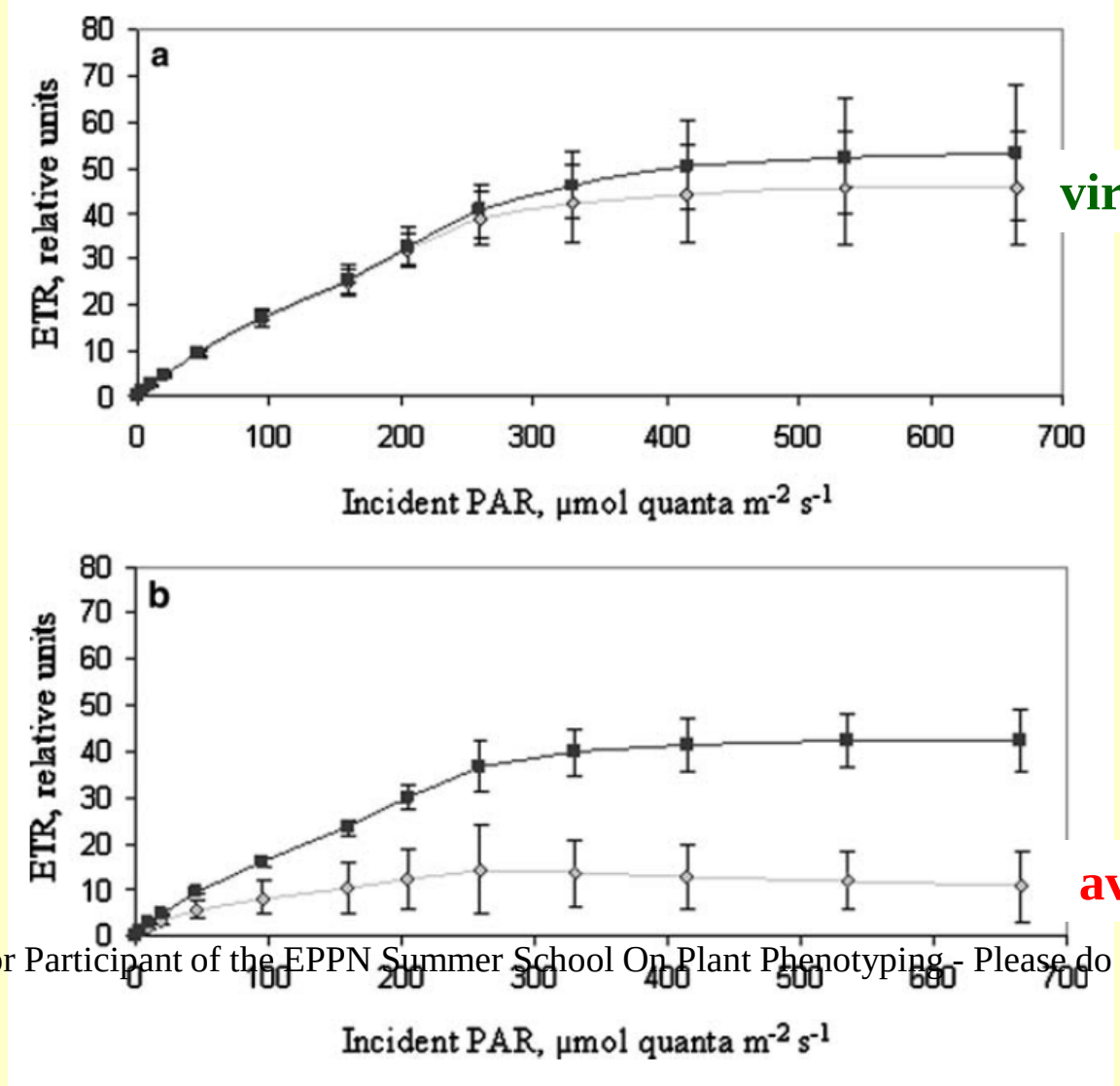
Bonfig (2006) Planta 225: 1-12

Differential effect of virulent and avirulent strains of PST on chlorophyll fluorescence parameters



maximum PS II quantum yield, F_v/F_m, effective PS II quantum yield, Y(II), and the coefficient of nonphotochemical quenching, qN. The im-

Differential effect of virulent and avirulent strains of PST on light response curves



virulent

avirulent

Effect of infection on excitation energy Flux in PS II

Loss through 3 fundamentally different pathways:

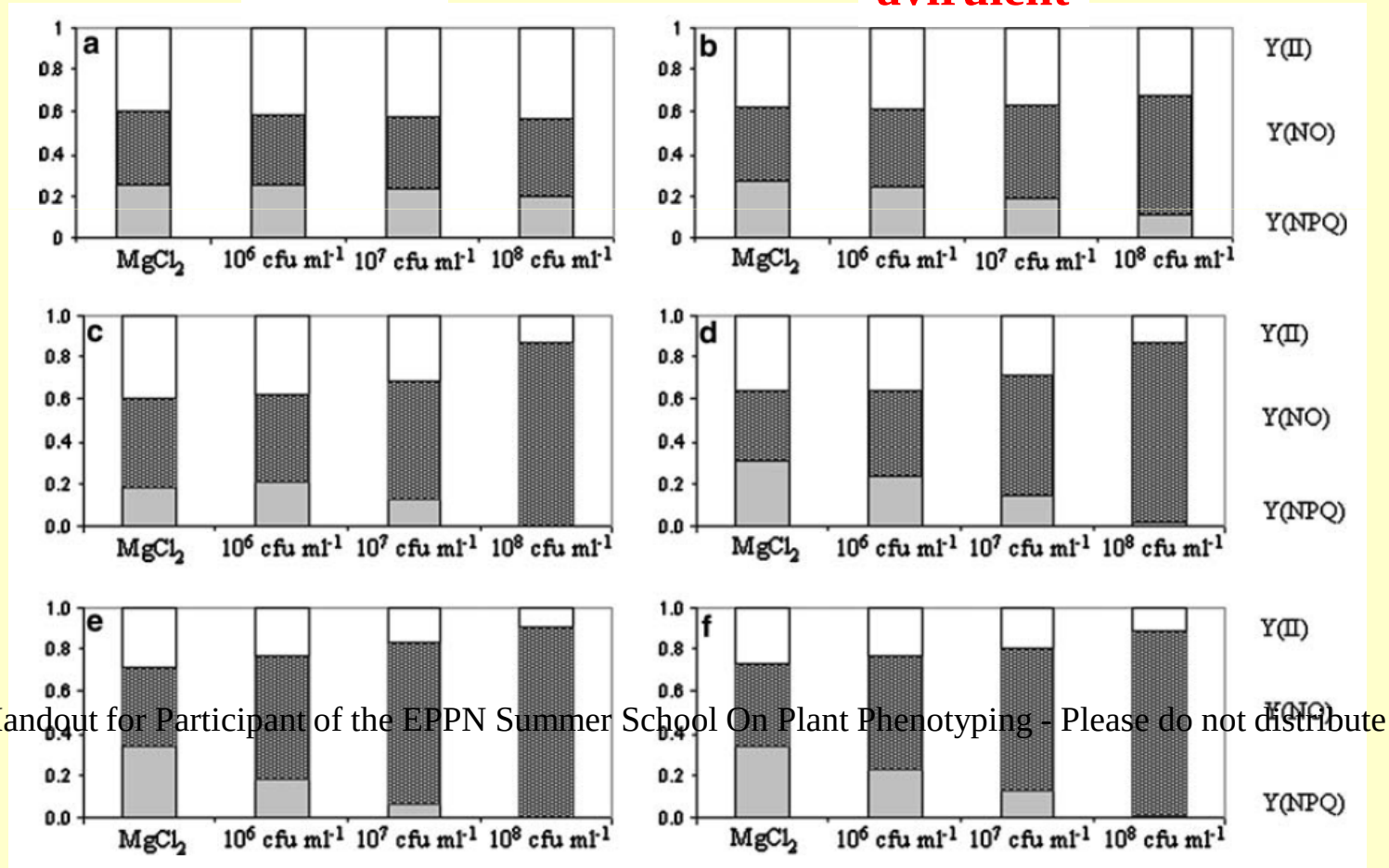
- Photochemical Utilization: quantum yields Y(II)
- Regulated heat dissipation (loss process serving for protection): Y(NPQ)
- Nonregulated heat dissipation (a loss process due to PS II inactivity): Y(NO)

virulent **avirulent**

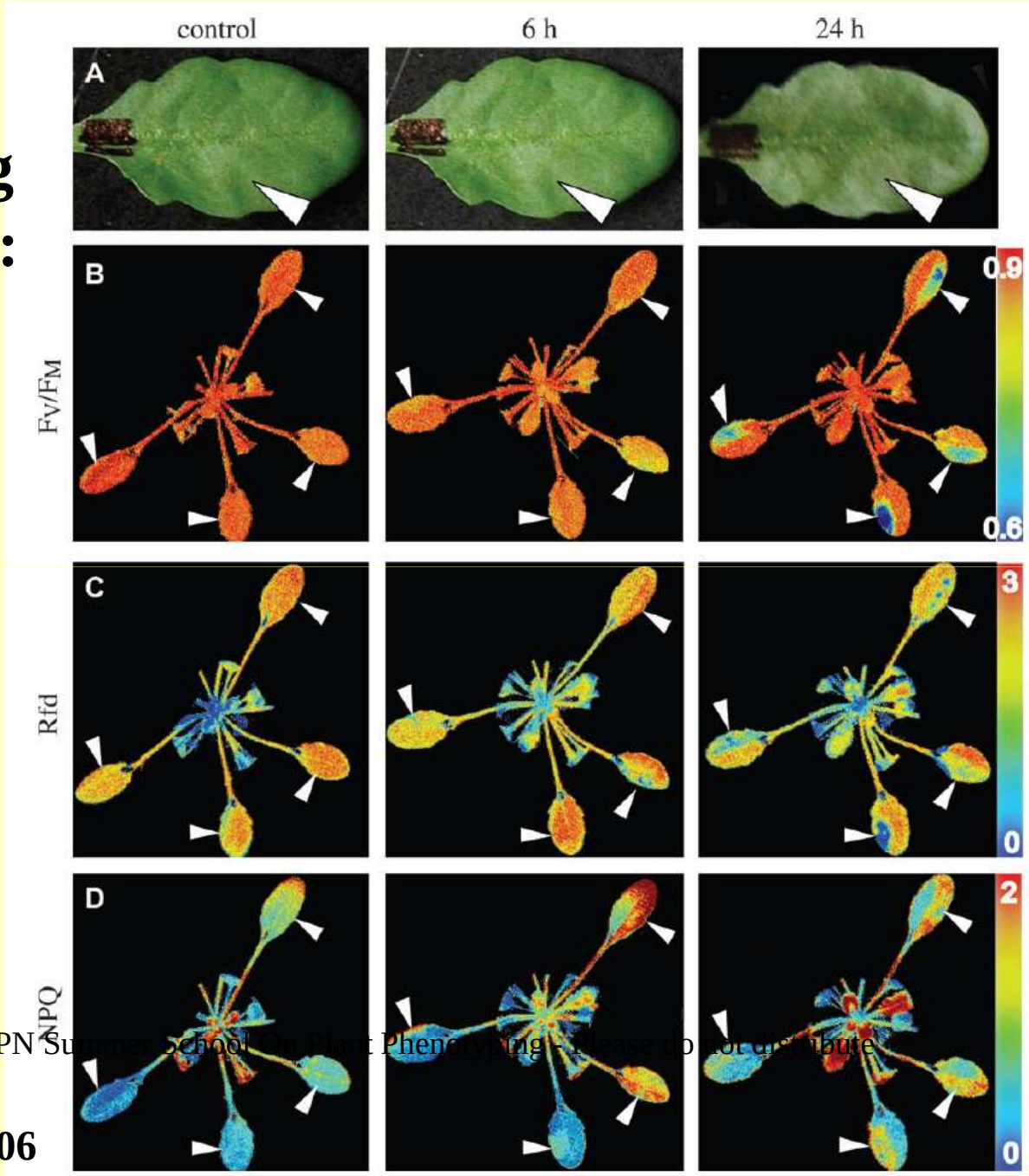
3h

24h

48h



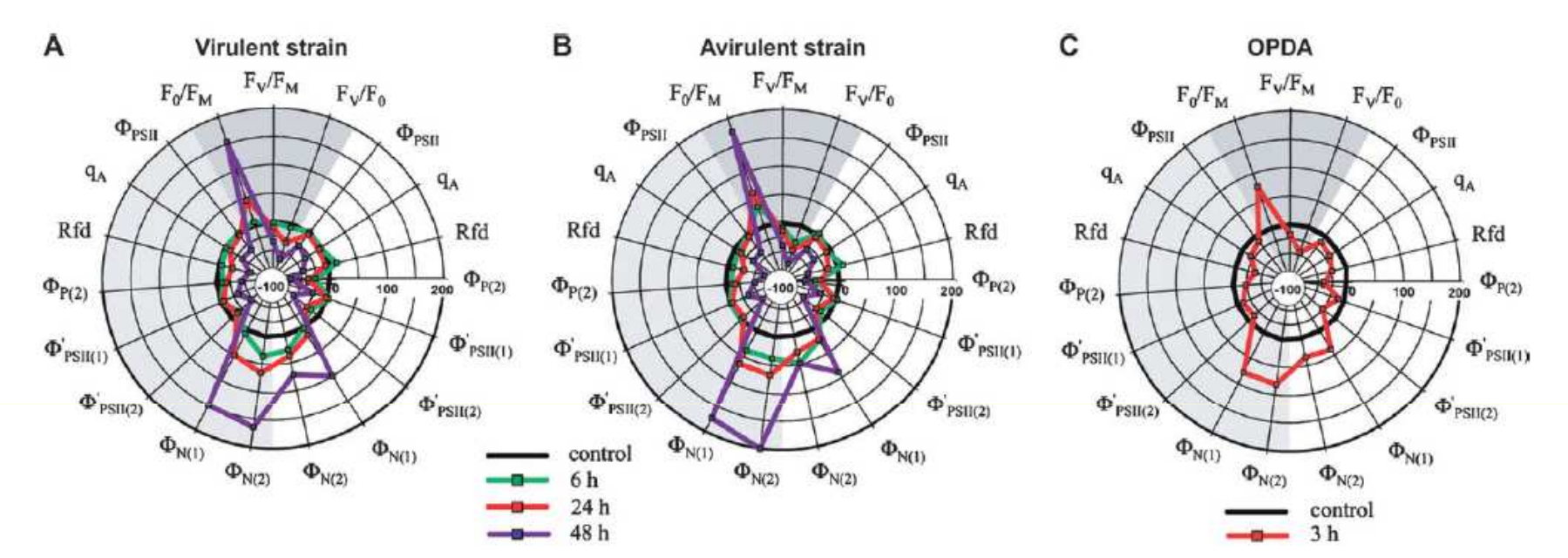
Combination of chlorophyll fluorescence imaging & statistical analysis:



Rfd
Fluorescence decline ratio
induced by 200 umol m-2 s-1
Photosynthetic capacity

Berger (2007). J. Expt. 58: 797-806

Differential effects of oxylipin treatment and Pst infection on chlorophyll fluorescence fingerprints



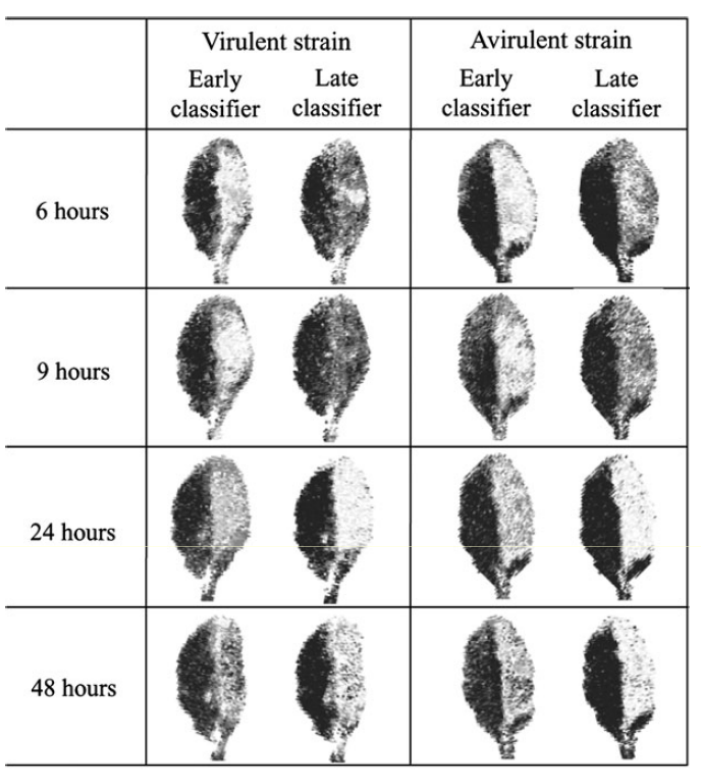
Relative changes of mean values of 19 selected standard fluorescence parameters described in literature of A. *thaliana* infected by *P. syringae* or treated by signaling compound and displayed in spider diagramms

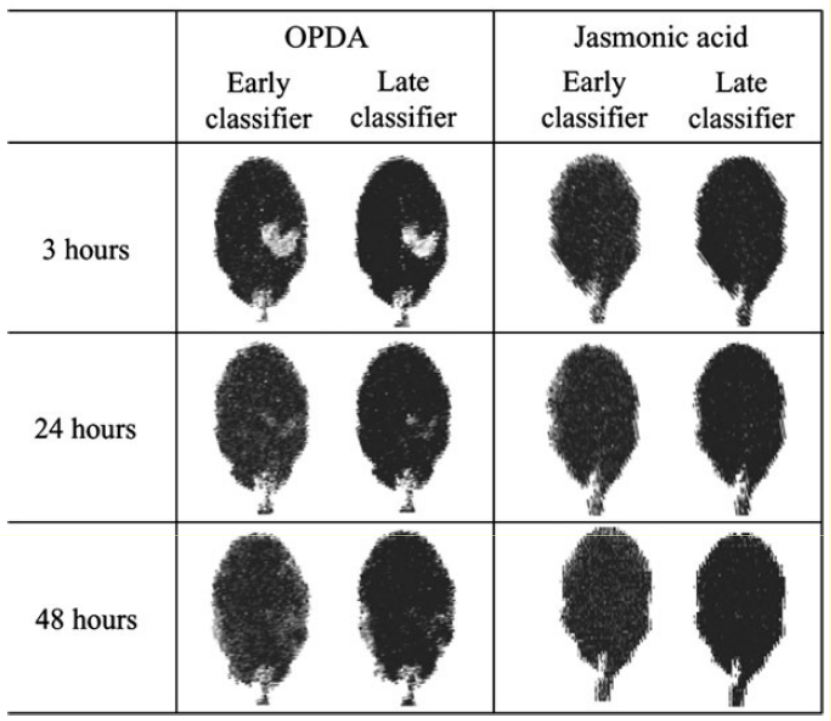
- Lightly shaded: low actinic light
- Non-shaded: high actinic light.
- Not shown: harmonically modulated light.

Discrimination:

- Time of infection: early - late
- Signalling compound (SA/JA - OPDA

Combinatorial fluorescence imaging

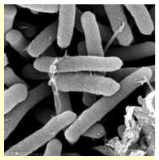


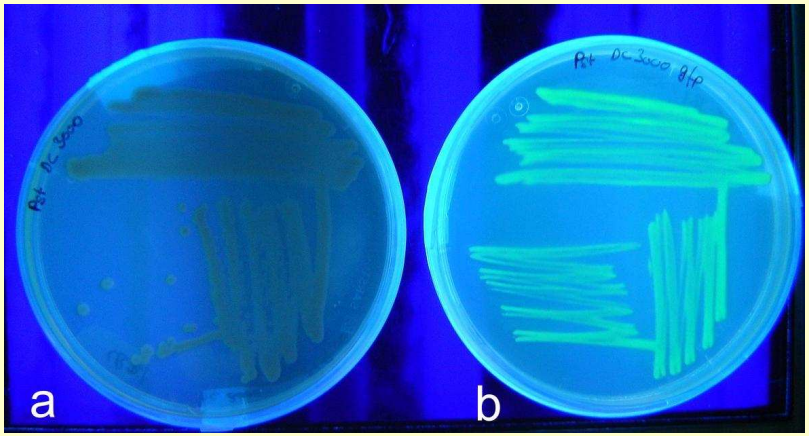


The images generated by classification of individual pixels for their relative resemblance to fluorescence transients affected by 9h (Early classifier) and by 24h (Late classifier) of infection by the virulent strain of P. syringae.

Basis: A complex experimental protocol was applied with 331 independent fluorescence images capturing Kautsky induction and quenching analysis in 50 and 200 umol m2 s-1 and six transients in fluctuating, harmonically forced light (T=1.5, 3, 6, 12, 24, and 48 s) with two amplitudes 80 and 160 umol m-2 s-1

Labelling of *P. syringae* with the autofluorescent green fluorescent protein (GFP)





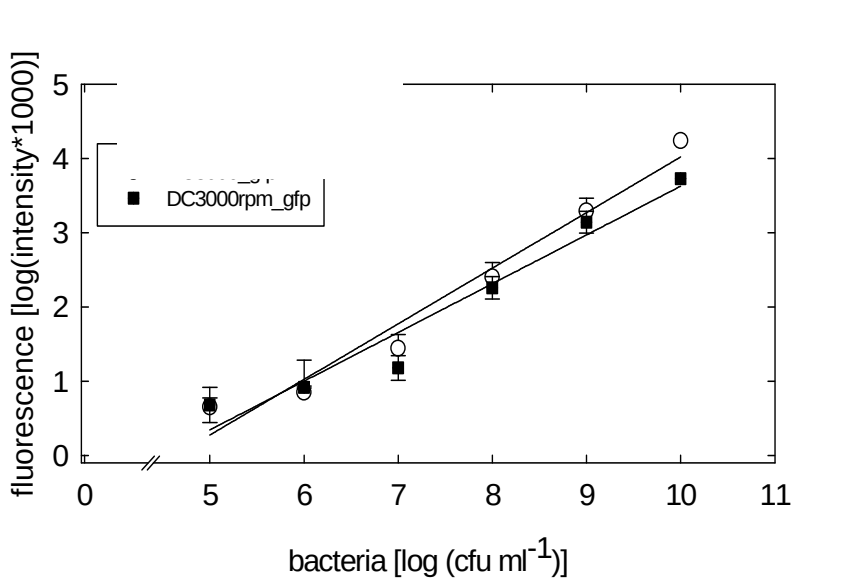
UV light illumination of *P. syrinage* DC3000 expressing GFP

Infection of *A. thaliana* with GFP expressing *P. syrinage* DC3000

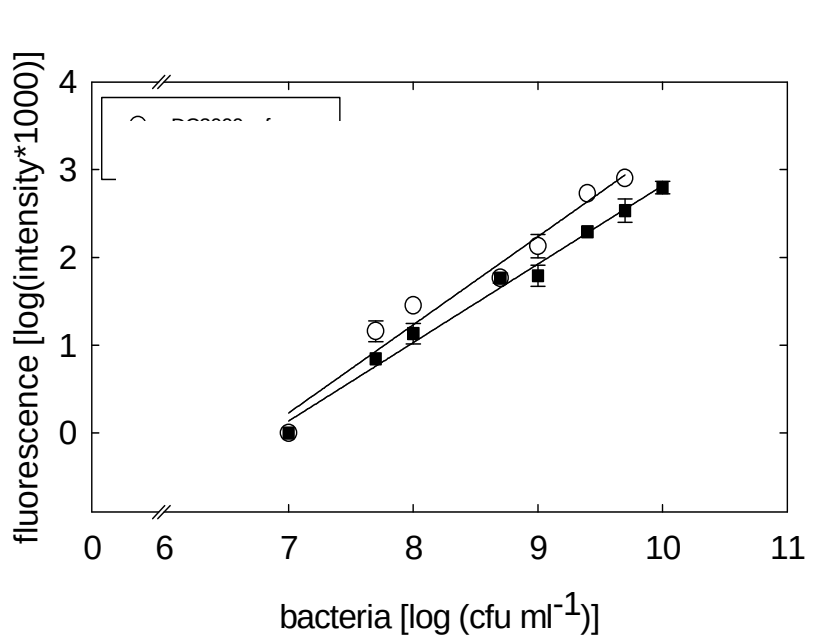
- Time course analyses with fluorescence microscope

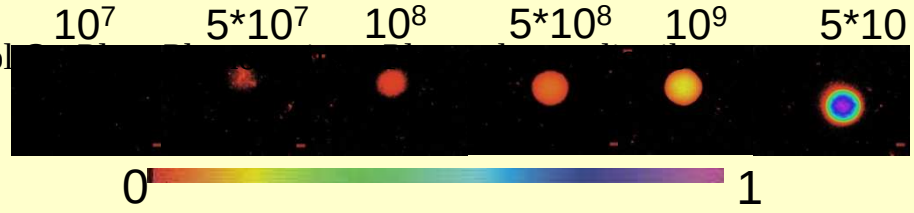
Quantification of *P. syringae* GFP fluorescence

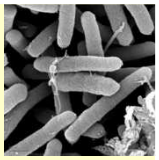
Fluorometer
(450 nm)



PAM/GFP measuring head
(450 nm)



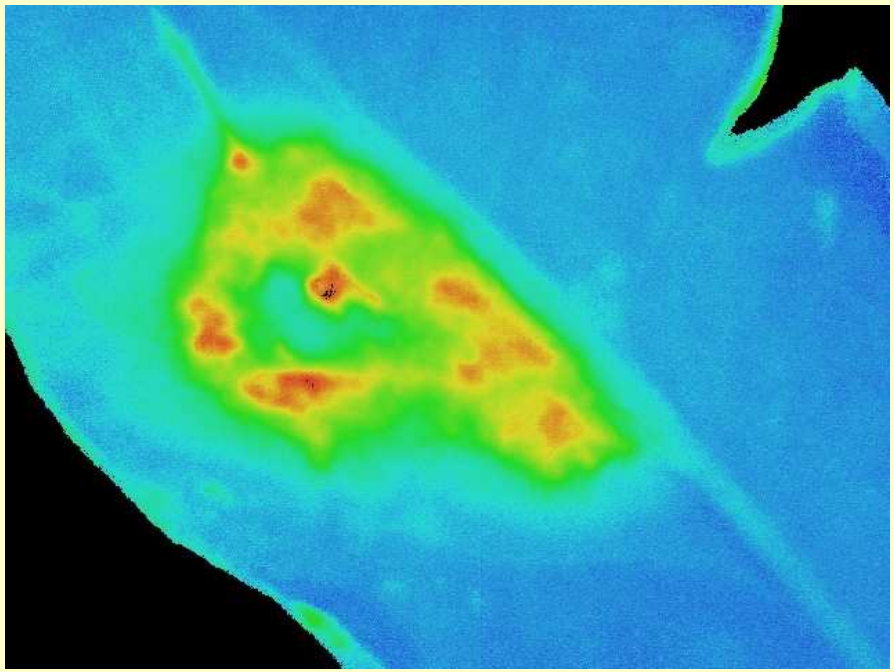




Combining Chlorophyll Fluorescence Imaging with detection of GFP labelled *P. syringae*

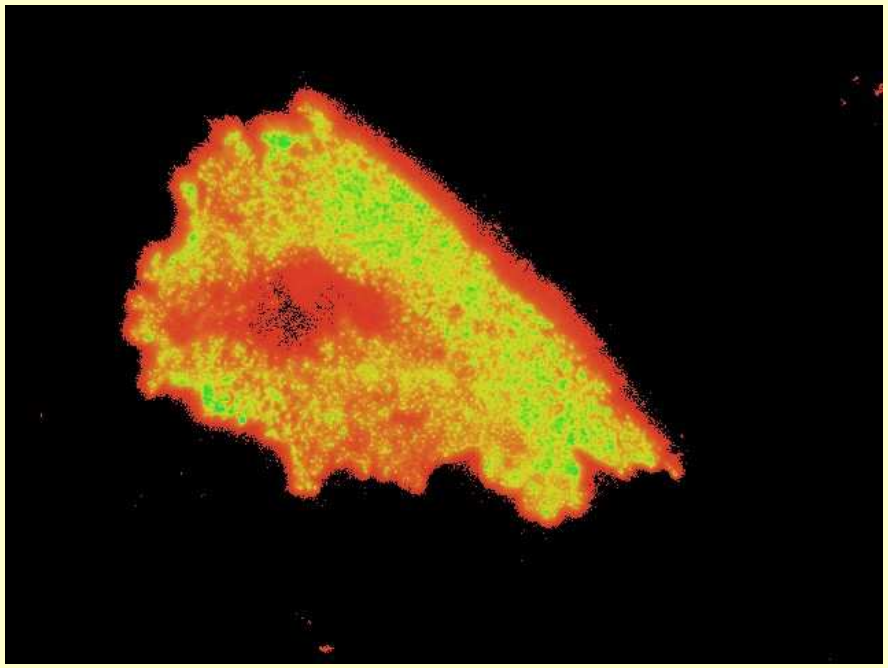
Analyses with custom made imaging system for determination of both chlorophyll and GFP fluorescence

Chlorophyll Fluorescence Imaging



Fv/ Fm

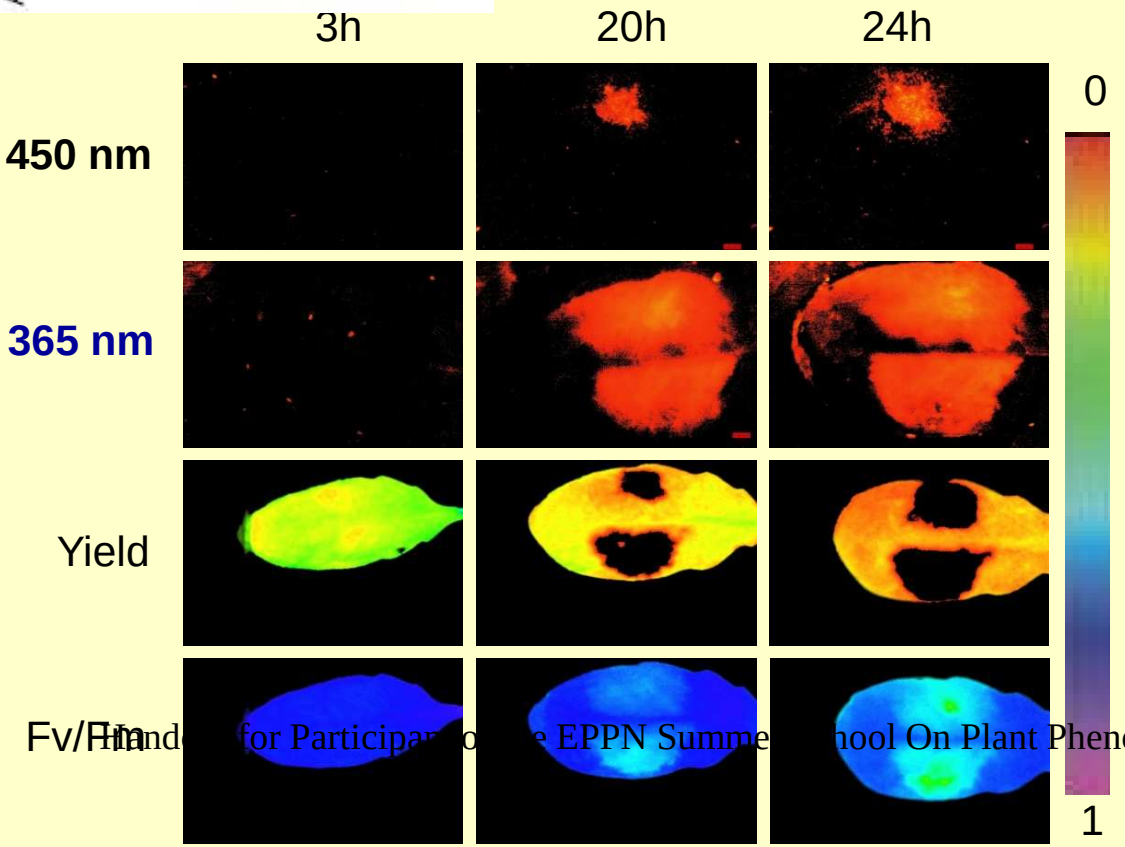
GFP Imaging (450nm)



F

Discrimination between phenolic and GFP fluorescence

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



3. Sucrose metabolism and abiotic stress tolerance

YOUNG LEAF = SINK:
Sucrose import

SOURCE LEAF:
Sucrose export

FRUIT = SINK:
Sucrose import

ROOT = SINK:
Sucrose import

SALT/DROUGHT STRESS

***Hypothesis*:**

The regulation of source-sink relationships:

•adapt to the new conditions

•continue to generate new resources and new growth

to minimising the impact of the stress.

Vigour maintenance

Delay of senescence

New biomass partitioning

Abiotic stress tolerance

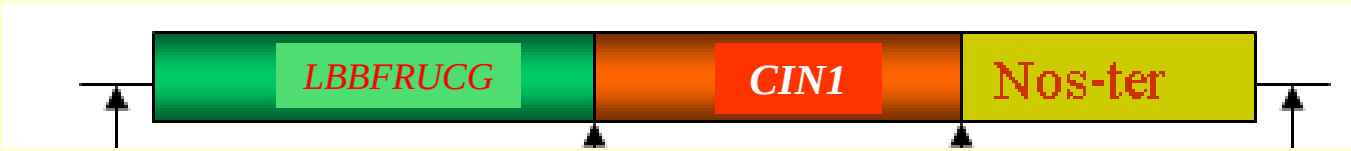
?

Metabolic factors: sucrose metabolism

Hormonal factors: **CKs**, Auxins, ABA, ACC

Francisco Pérez-Alfocea et al., CEBAS-CSIC, Murcia

Ectopic overexpression of extracellular, cell wall invertase CIN 1 in tomato

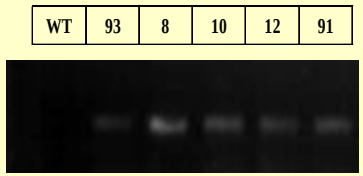


Assumed fruit specific promoter

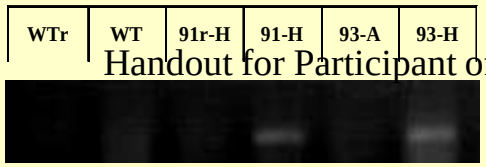
CIN1* = cell wall invertase from *Chenopodium rubrum

The CIN1 transgene is expressed in various tissues during development

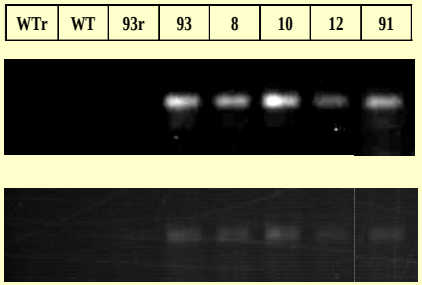
SEEDLINGS



FRUITS

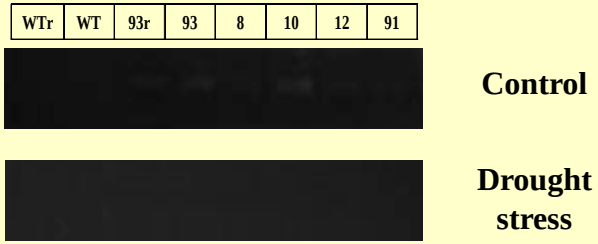


LEAF



ROOT

ROOT



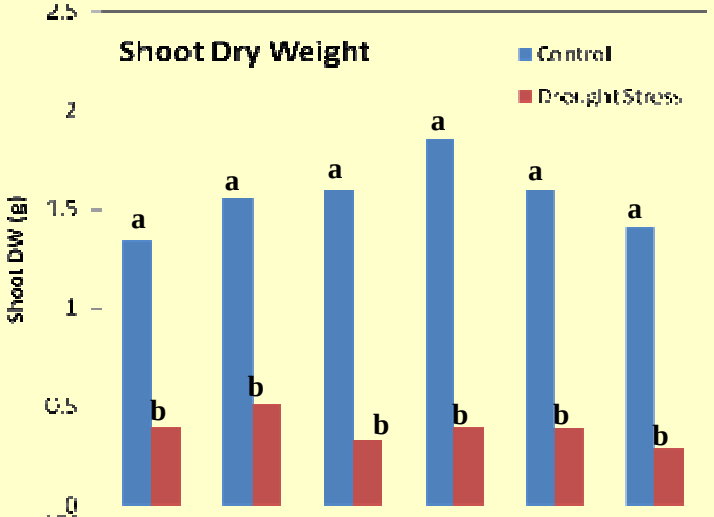
Albacete et al., in revision

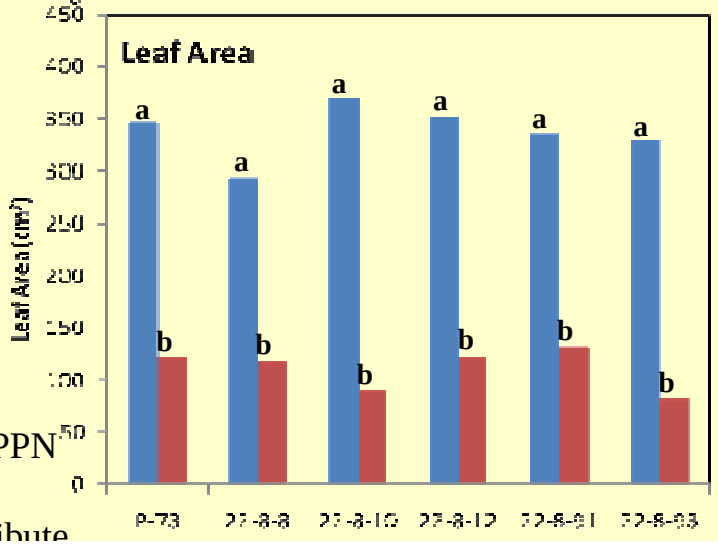
CIN1 overexpression: drought stress tolerance

- **Extreme drought tolerance in CIN1 plants**

Drought stress tolerance in *CIN1* plants are not due to differences in growth parameters

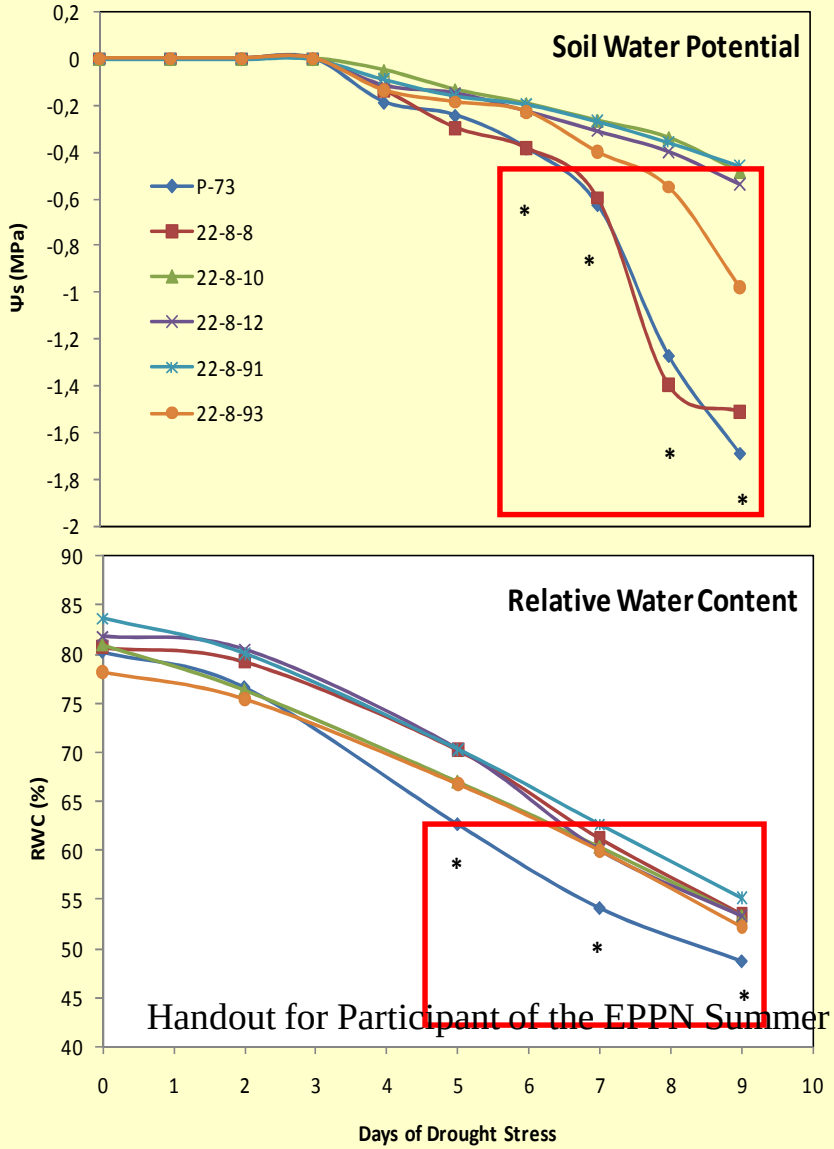
WT **CIN1 1-91**





CIN1 overexpression: drought stress tolerance

Soil water potential and relative water content improved in *CIN1* plants

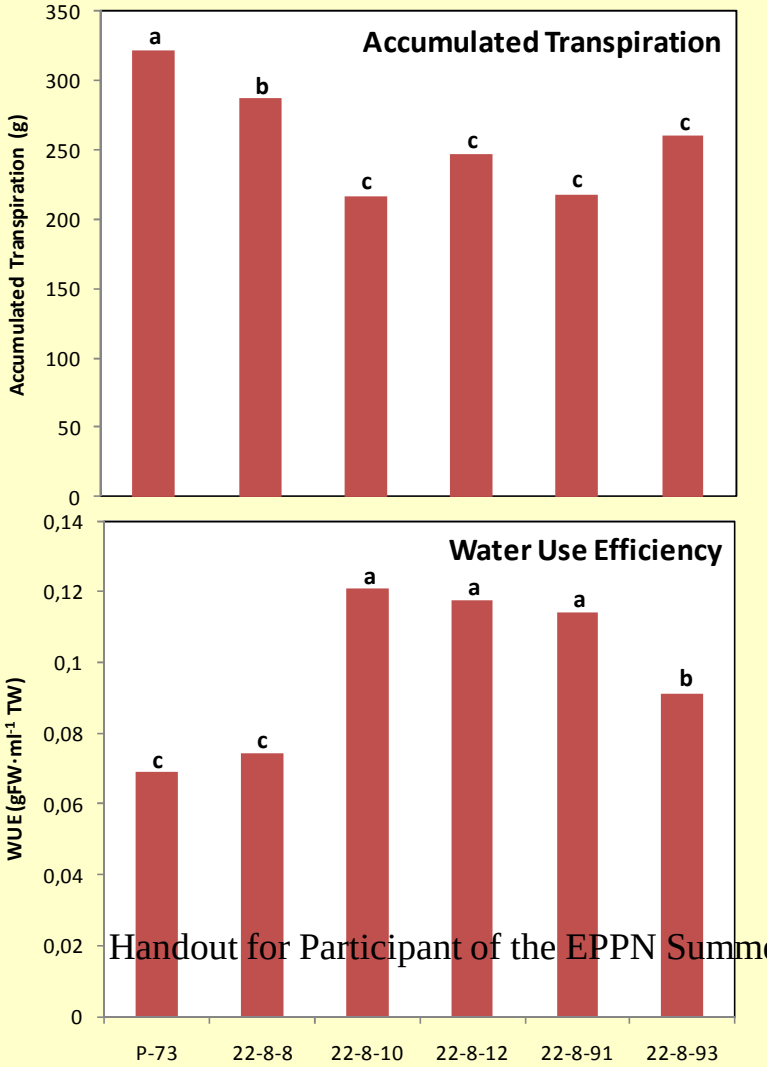


wt plants dried the substrate faster than *CIN1* plants

RWC was severely reduced during drought stress in wt

CIN1 overexpression: water use efficiency

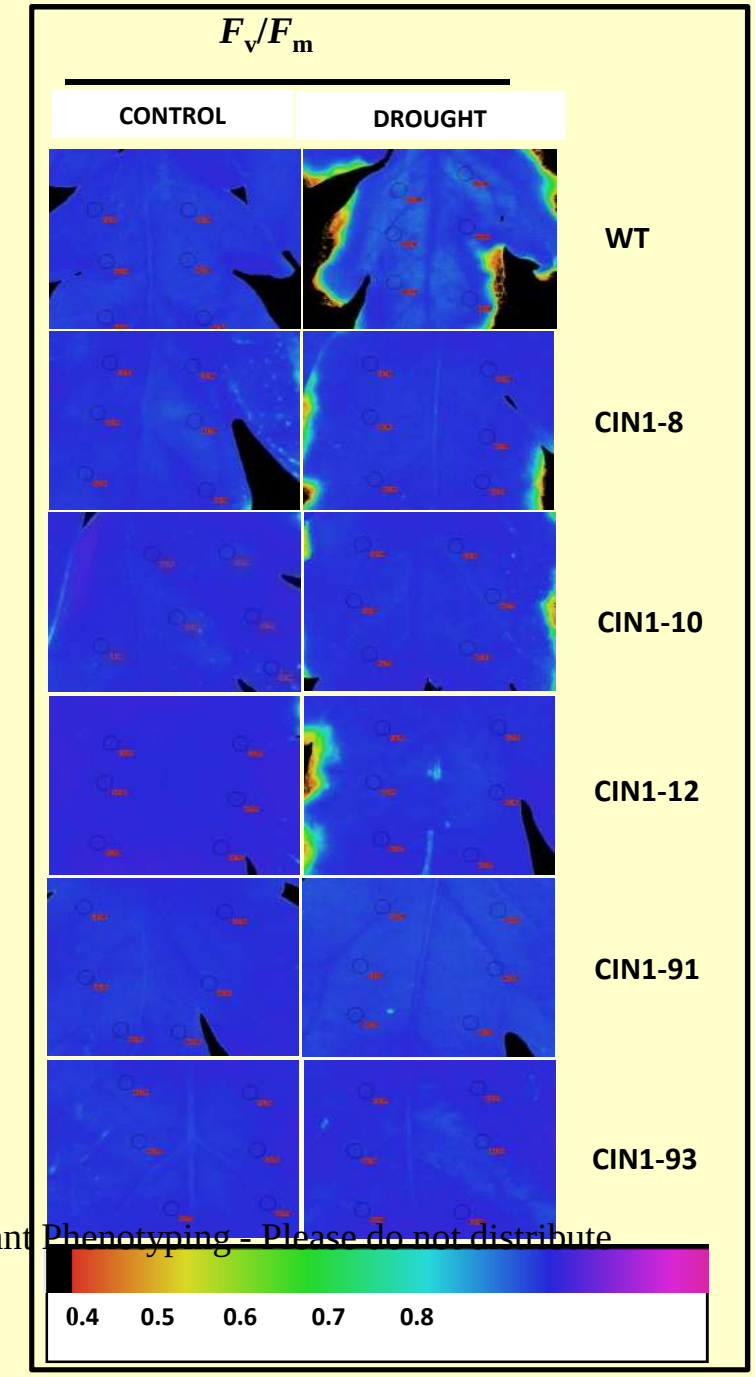
- **Water use efficiency (WUE) is improved in *CIN1* plants under drought stress**



Accumulated transpiration during drought was significantly lower in *CIN1* plants.

WUE (measured as the **amount of generated biomass per ml of transpirated water**) was higher in *CIN1* plants.

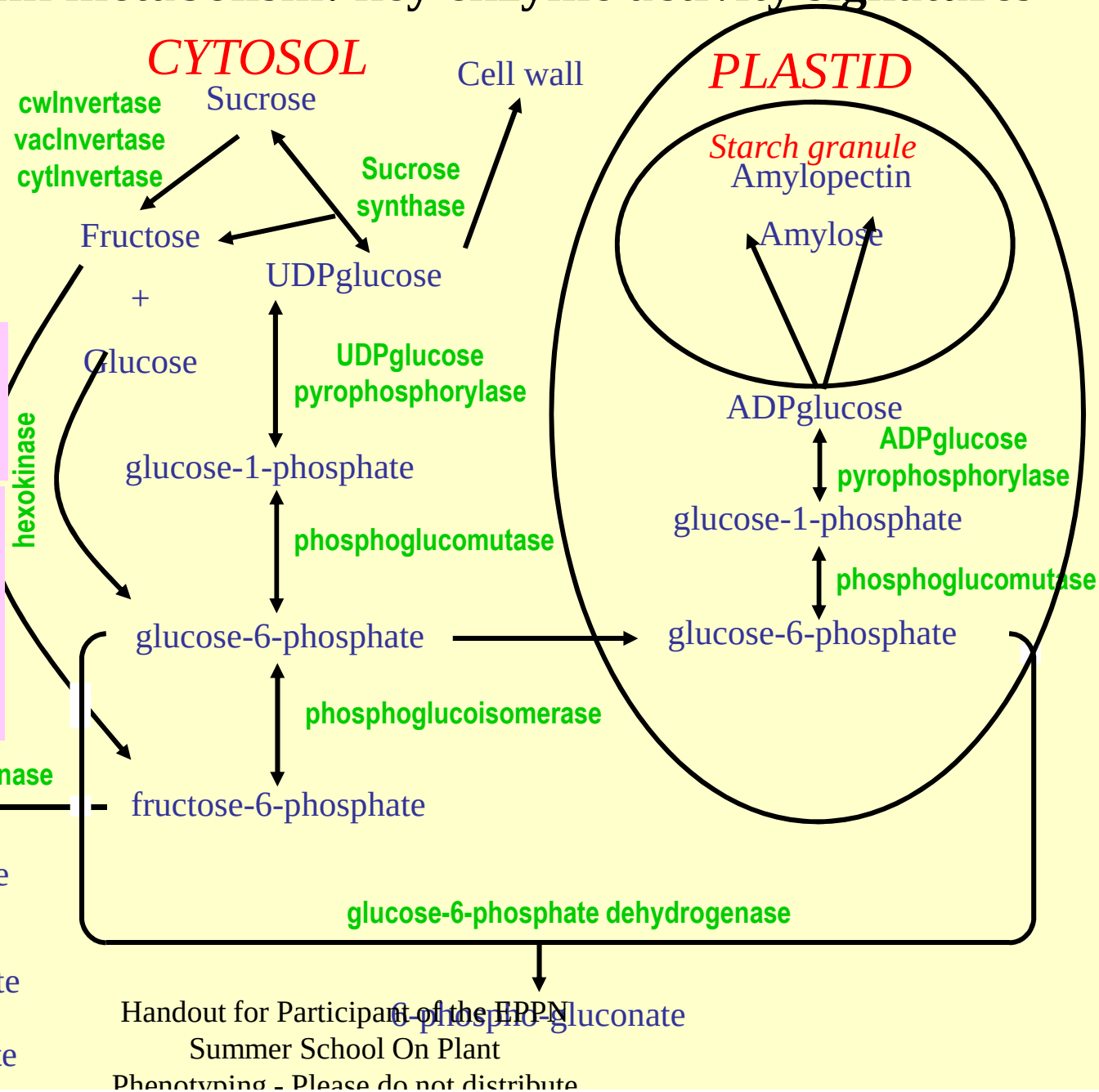
CIN1 overexpression: chlorophyll fluorescence



Carbohydrate sink metabolism: key 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



CIN1 overexpression: enzyme activity signatures

Sucrolytic- and invertase inhibitor activities in *CIN1* plants under drought

Assay	Signature under drought stress	control
invertase inhibitor	decrease in *CIN1*, increase in wt	lower activity in wt
vacuolar invertase	decreased activity	
cell wall invertase	decreased activity	lower activity in wt
cytoplasmic invertase	decreased activity	
Susy	decreased activity	higher activity in wt
Aldolase	decreased activity	
Glucose-6 phosphate dehydrogenase	weak decrease in *CIN1*, strong decrease in wt	higher activity in wt
Hexokinase	increase in *CIN1*	
phosphofructokinase	increase in *CIN1*, decrease in wt	higher activity in wt
phosphoglucoisomerase	increased activity	higher activity in wt
phosphoglucomutase	no change in *CIN1*, decrease in wt	higher activity in wt
UGPase	no change in *CIN1*, decrease in wt	higher activity in wt
Fructokinase	no change in *CIN1*, decrease in wt	higher activity in wt
AGPase	not detectable	not detectable

Sugar contents:

-control conditions, increased fructose and glucose levels in *CIN1* plants

-drought stress, no specific changes

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)**

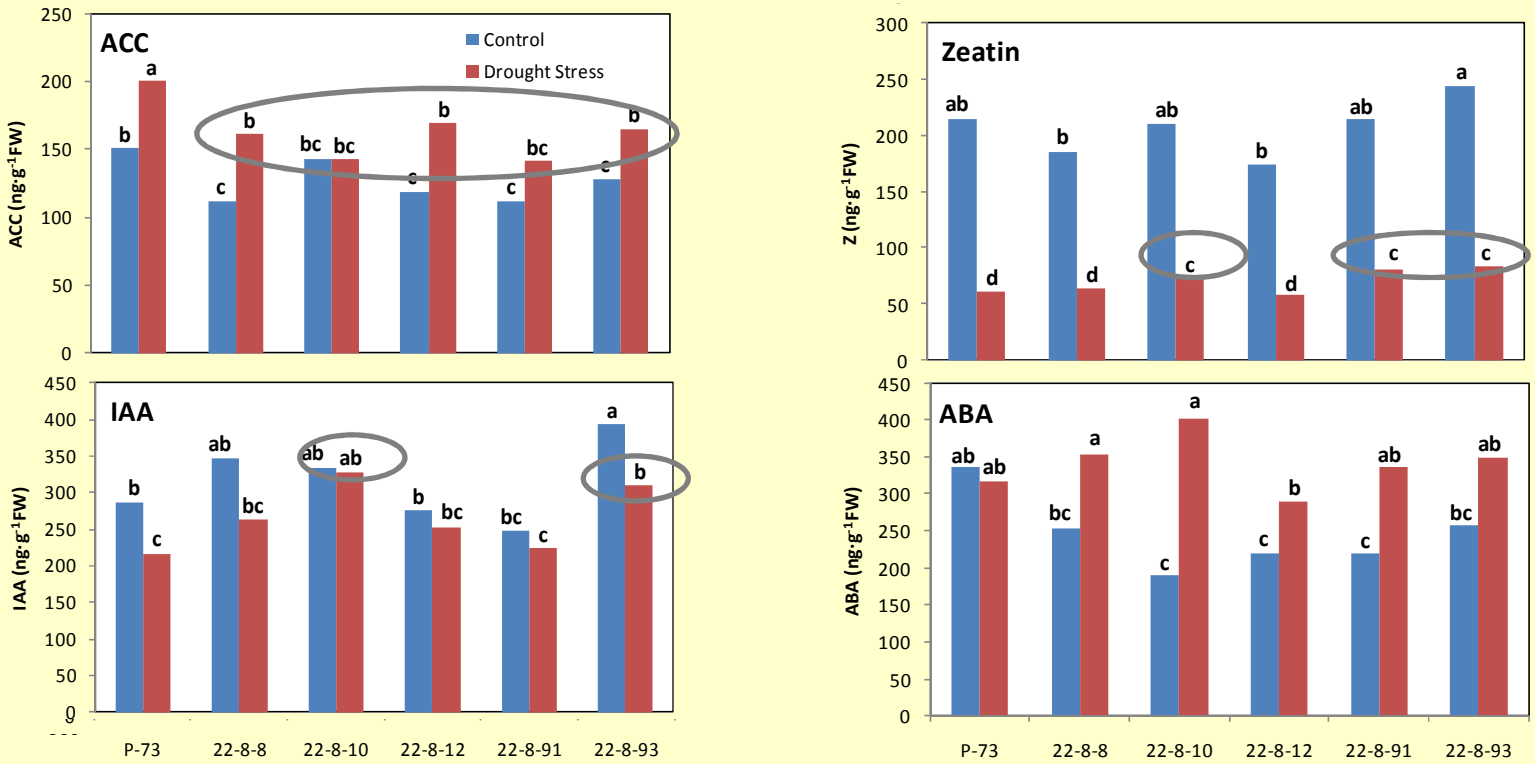
3) Currently established: Gibberellins

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

UHPLC/Ms-Ms

CIN1 overexpression: hormonal status

SUCROSE METABOLISM ⟷ HORMONAL FACTORS



- Decrease of the ET precursor ACC in *CIN1* plants under drought stress
- Increase in t-Zeatin for some *CIN1* lines under dro
- IAA also increased in some *CIN1* lines under drou
- No change in ABA levels!

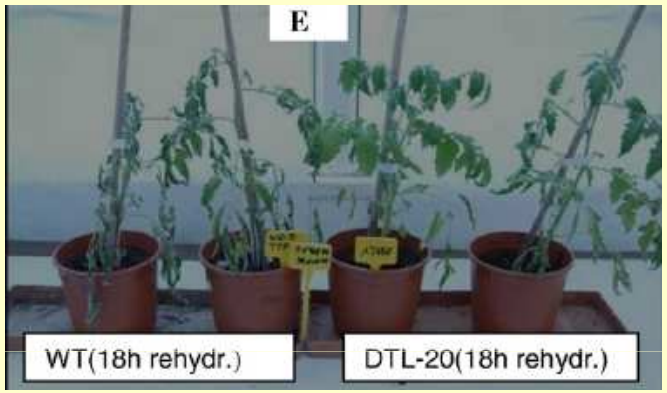
➢ **(3) Extracellular invertase contributes to abiotic stress tolerance**

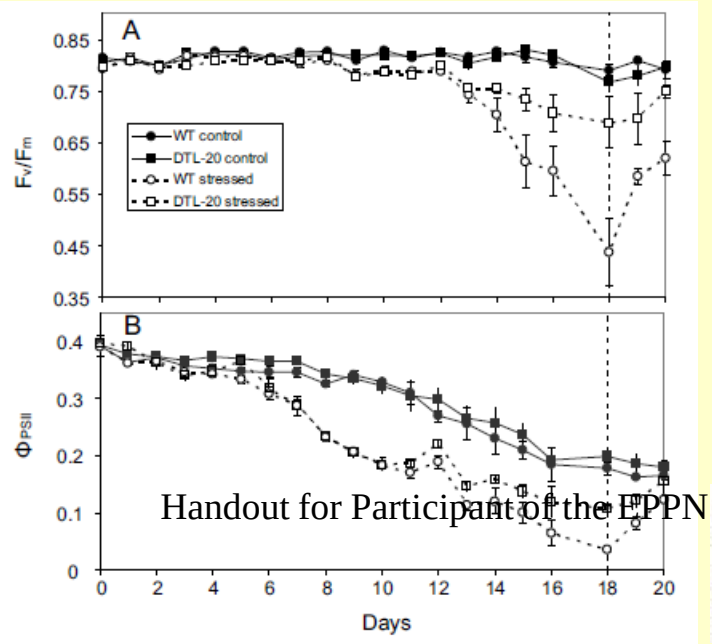
Engineered drought tolerance in tomato plants is reflected in chlorophyll fluorescence emission

Kumud Bandhu Mishra[a,*,1], Rina Iannacone[b,1], Angelo Petrozza[b], Anamika Mishra[a,c], Nadia Armentano[b], Giovanna La Vecchia[b], Martin Trtílek[d], Francesco Cellini[b], Ladislav Nedbal[a]

Plant Science 182 (2012) 79–86

ATHB-7 (ABA) mediated drought tolerance





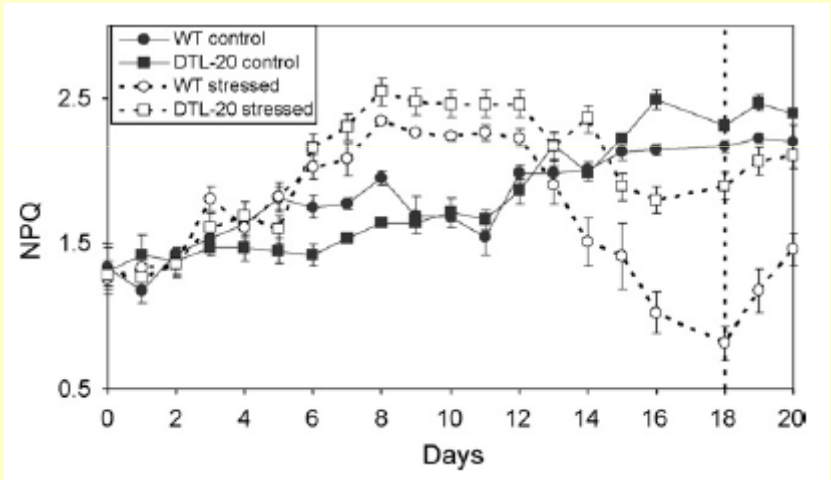
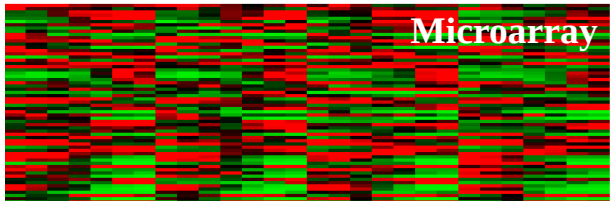


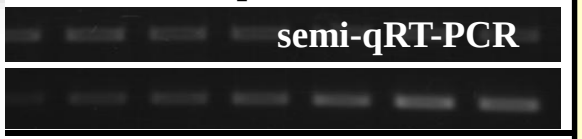
Fig. 6. Variation of non-photochemical quenching (NPQ) of WT and DTL-20 plants measured *in vivo* during the extended period (18 days) of drought and post re-hydration period (2 days). Vertical dashed line separates the drought and post-rehydration period. Values are mean ±SE ($n = 6$).

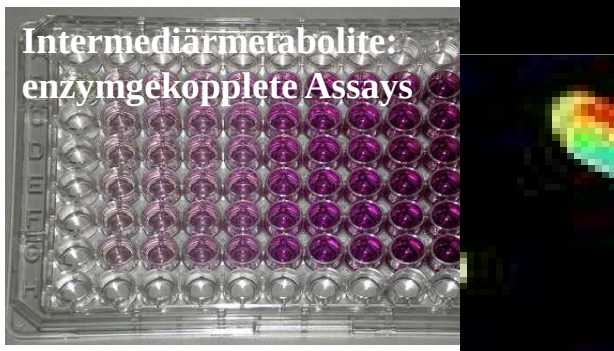
and (B) operating efficiency photosystem (PS) II (Φ_{PSII}) of WT and DTL-20 plants measured *in vivo* during the extended period (18 days) of drought and post re-hydration periods (2 days). Vertical dashed line separates the drought and post-rehydration period. Values are mean ±SE ($n = 6$).

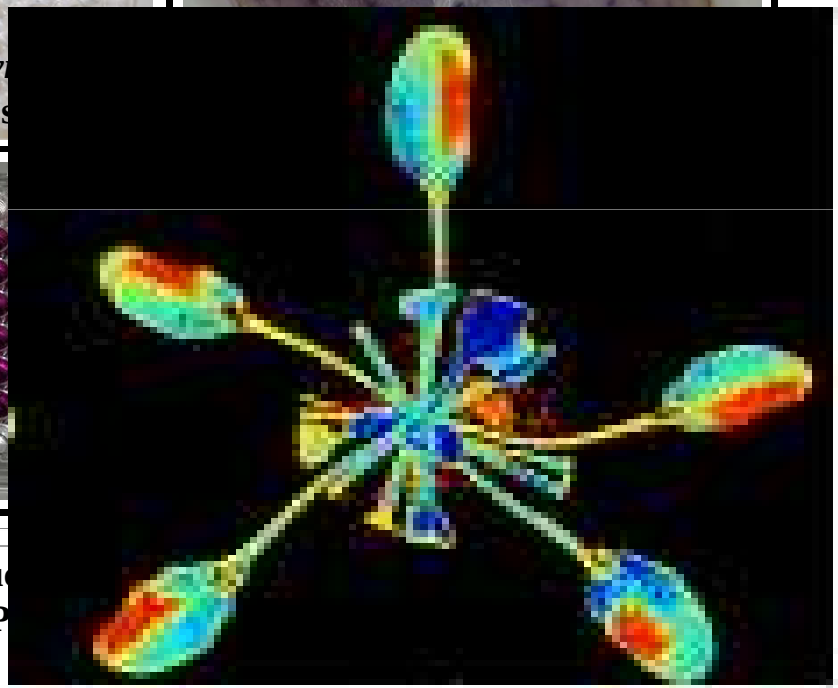
4. Integration of fluorescence signatures with physiological phenotyping: PHENOMICS „Plant physiology meets biophysics“



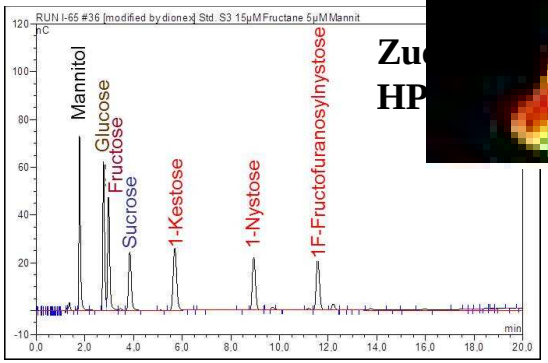
Genexpression



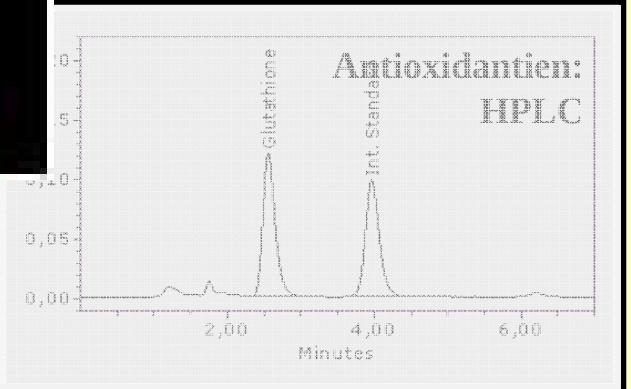


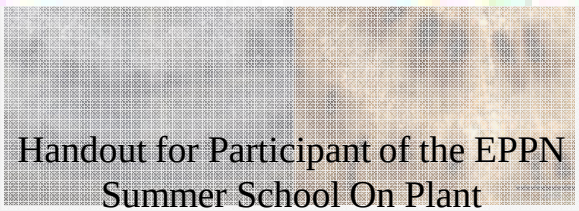


Phytohormone:
U-HPLC-HESI-MS/MS



Zu
HP



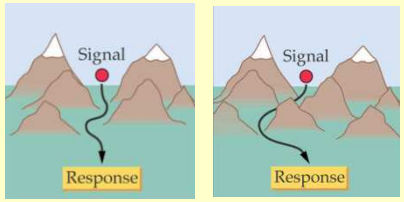


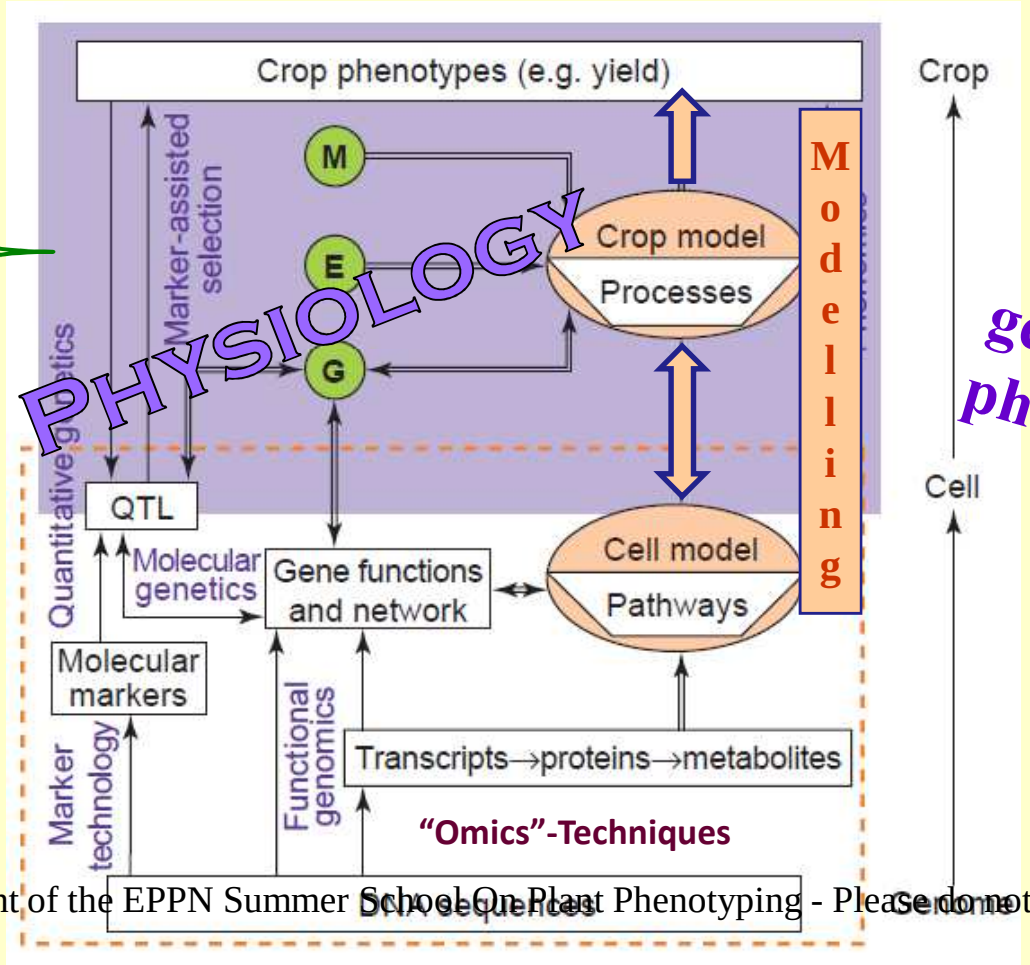
Phenotyping - Please do not distribute

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

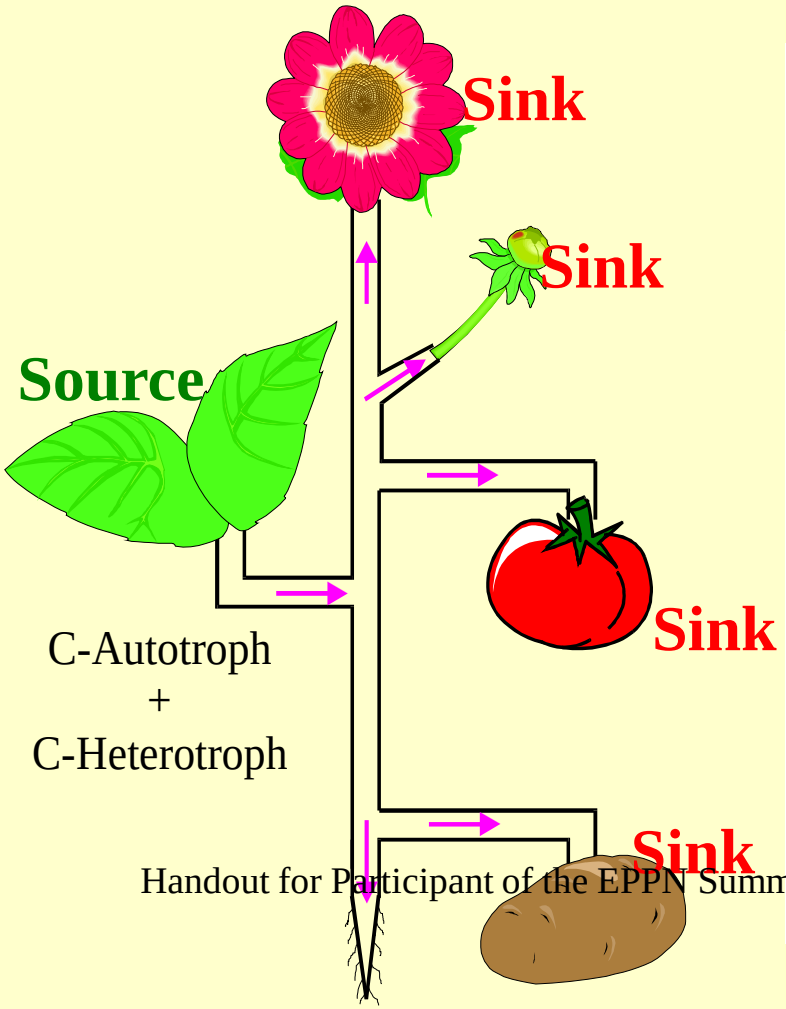




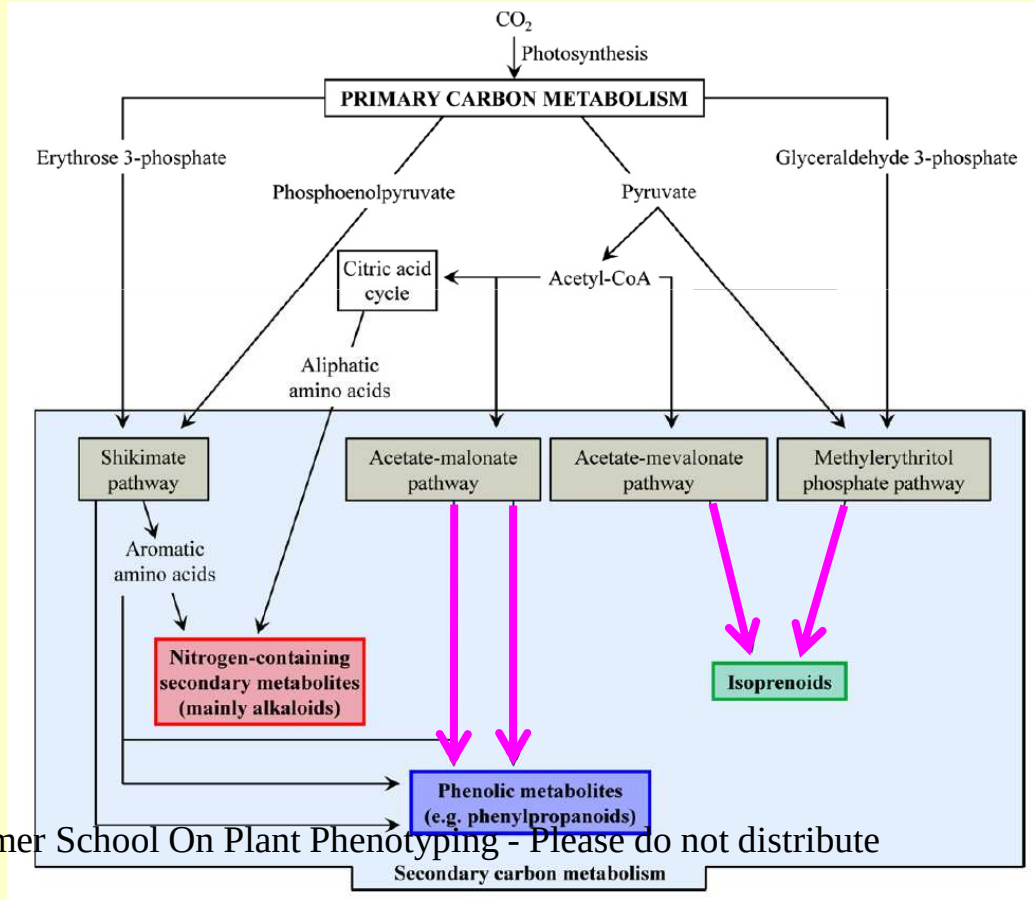
Yin, Struik & Kropff (2004) TIPS 9, 426-432. modified

Different scales of carbohydrate partitioning

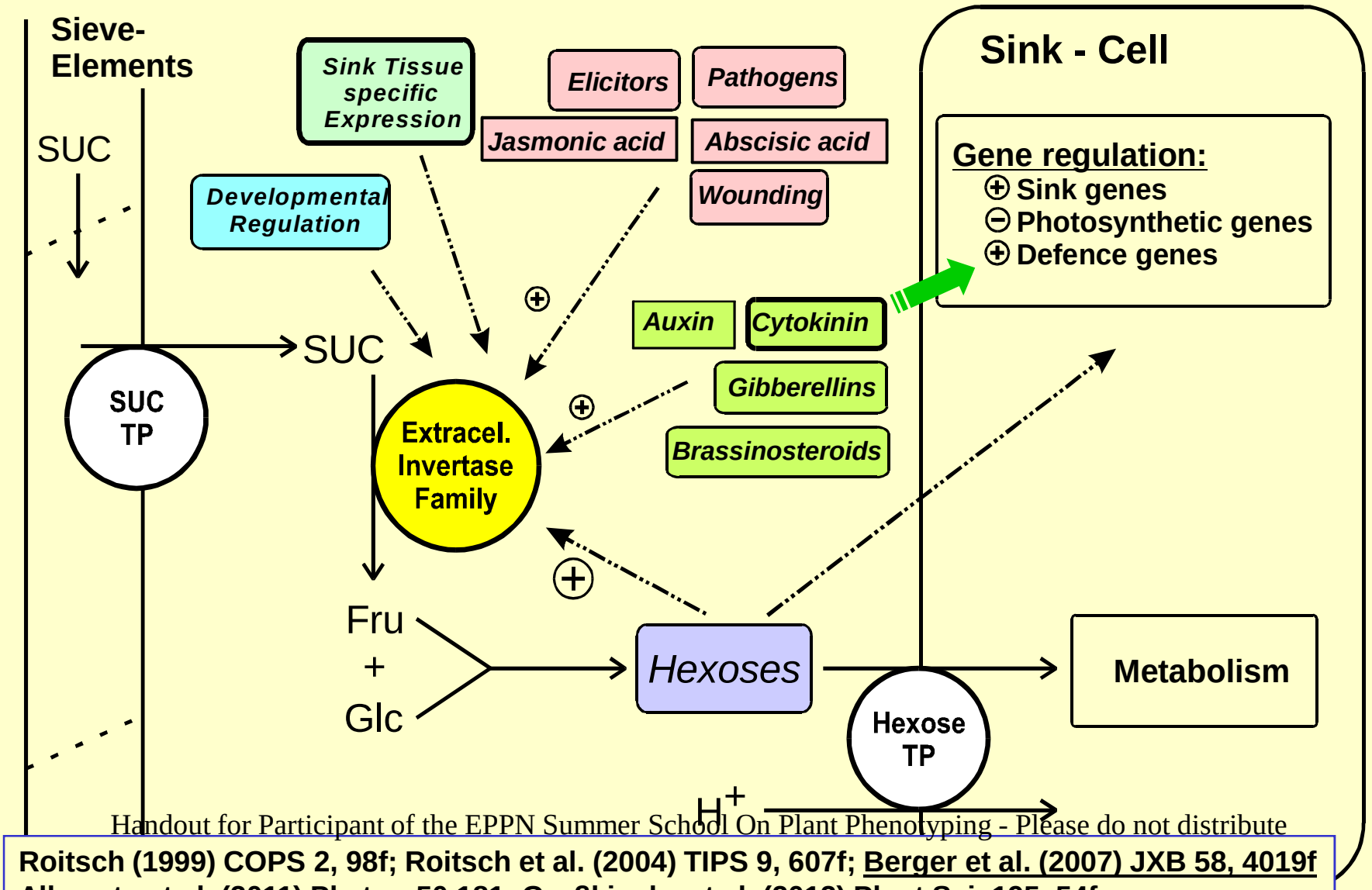
(A) Long distance transport from source and sink tissues



(B) Metabolic channeling from primary to secondary metabolism

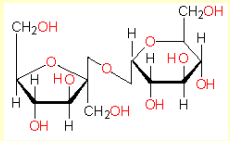


Extracellular Invertase: Key metabolic Enzyme and PR Protein



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

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



Sucrose

Glucose

Stress/Pathogen related stimuli

Calcium-Signatures

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

Acknowledegements

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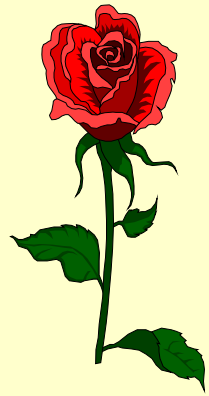
Martina Papadopoulos
Eric van der Graaff

Köszönöm!



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