UNIVERSITY OF COPENHAGEN

Physiological phenotyping to identify predictive markers and breeding targets

Kristian Thorup-Kristensen & Thomas Roitsch

1. Integration of physiological phenotyping into plant phenomics to assess the G x E x M interaction & close the genotype – phenotype knowledge gap
2. Central role of resource allocation for growth and stress responses
3. Physiological fingerprinting of carbohydrate metabolism via the determination of enzyme activity signatures
4. Preliminary results from the Robusta project

Department for Plant and Environmental Sciences
Copenhagen Plant Science Center
Molecular Plant Physiology and Plant Phenomics

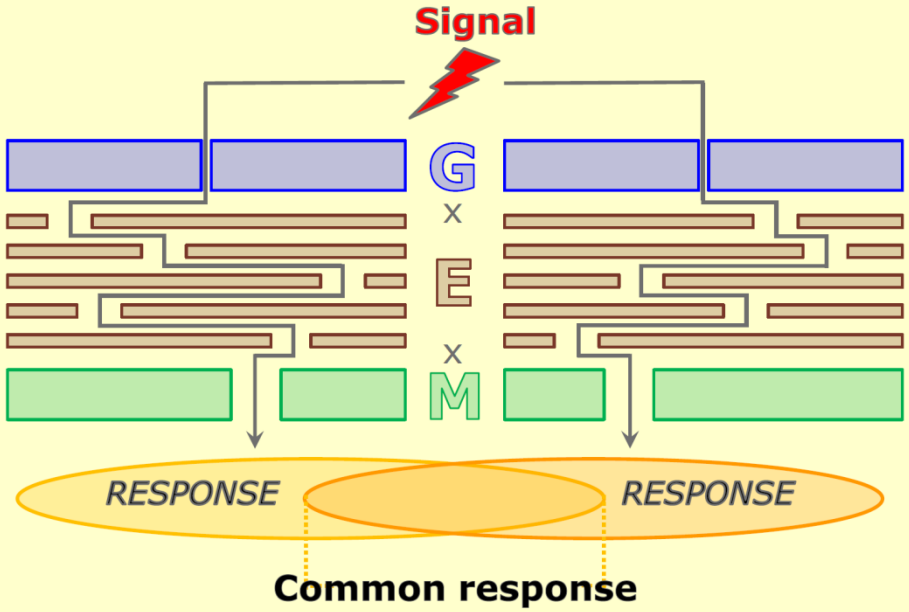
1. Plant phenomics - G x E x M - genotype/phenotype gap

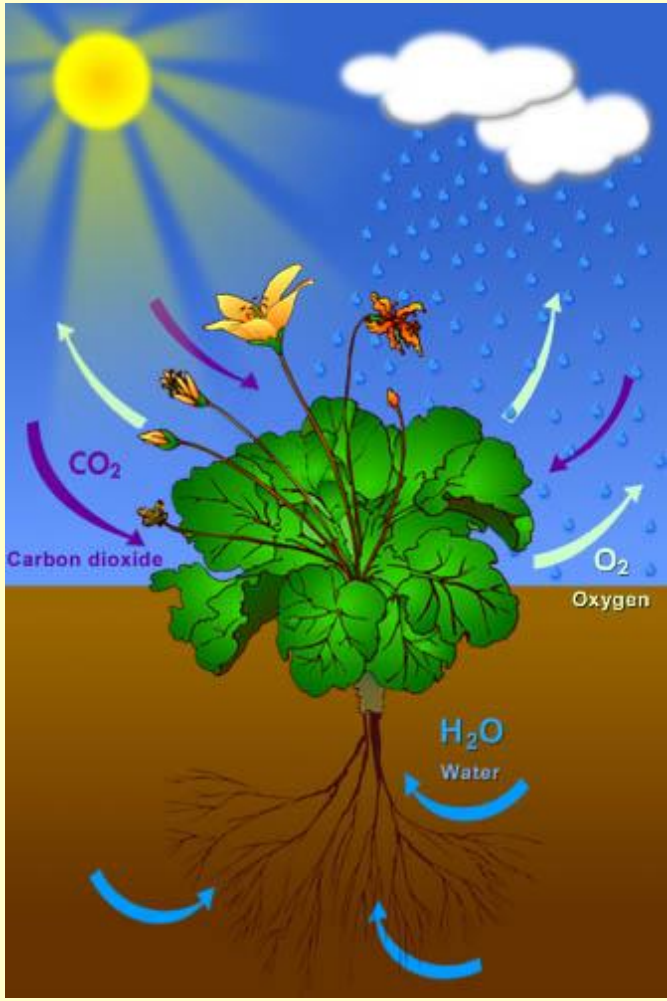
Phenotypic variation…

… is produced through a complex web of interactions between

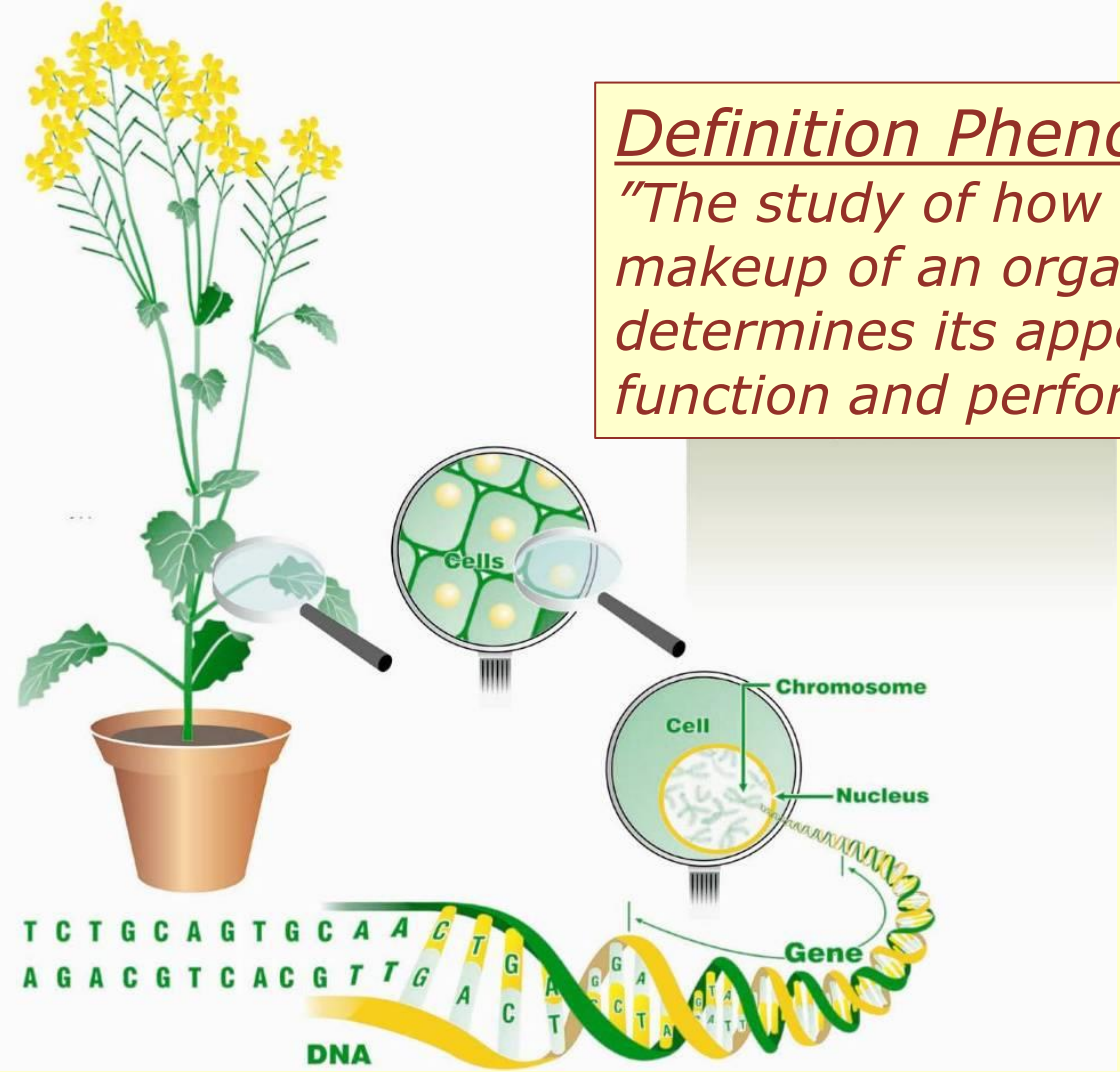
Genotypes x Environment x Management

…to ensure developmental programms and appropriate responses within variable external conditions





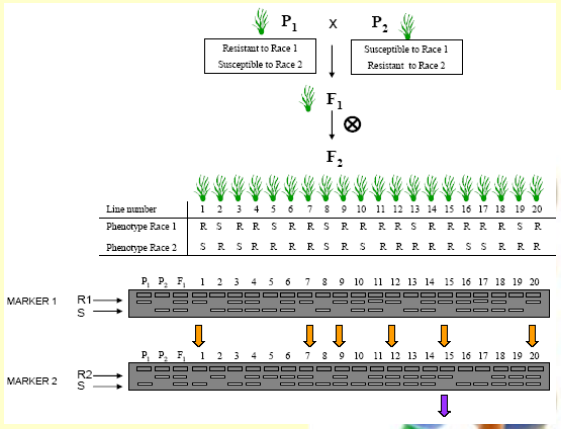
Plant phenomics

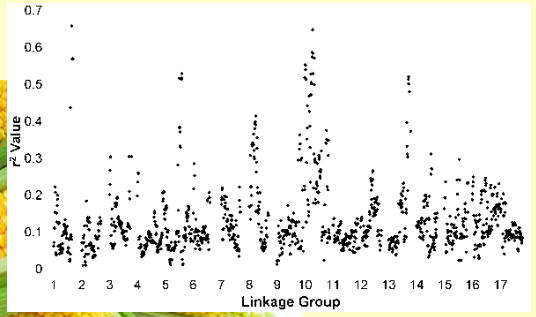


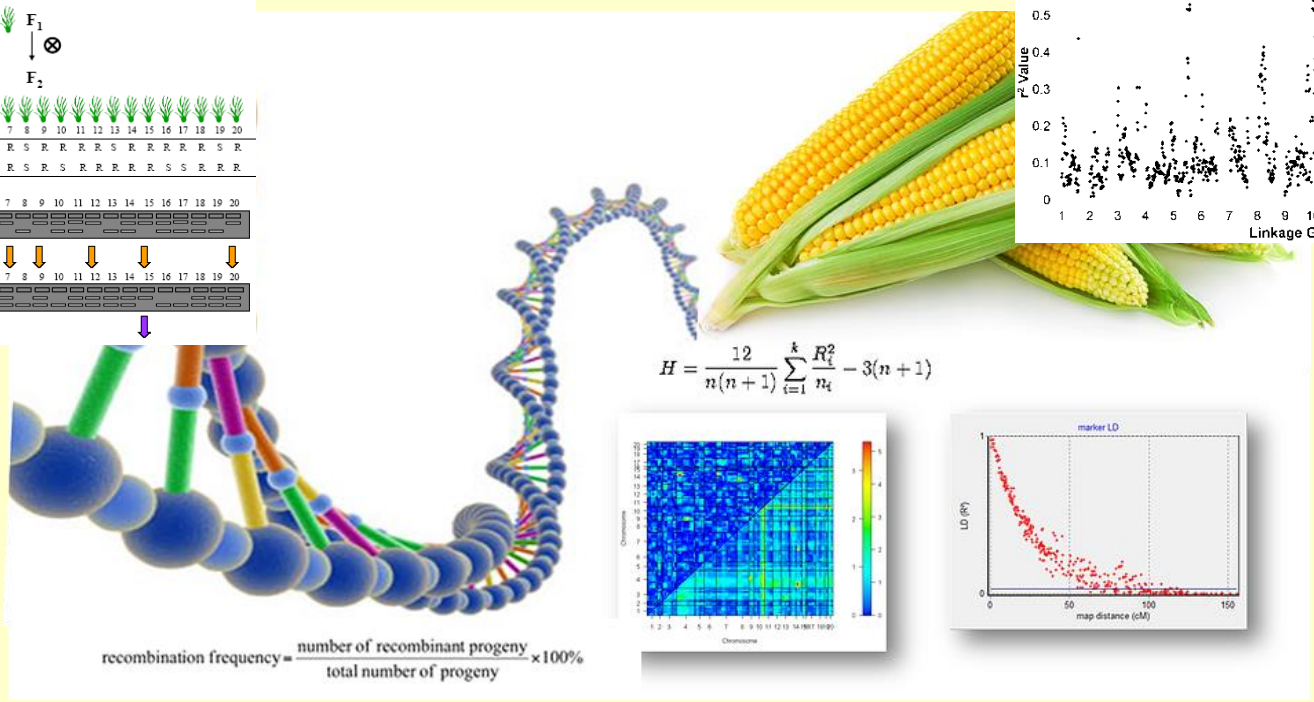
Definition Phenomics:

"The study of how the genetic makeup of an organism determines its appearance, function and performance"

Advances through high-troughput molecular breeding and non-invasive phenotyping methods

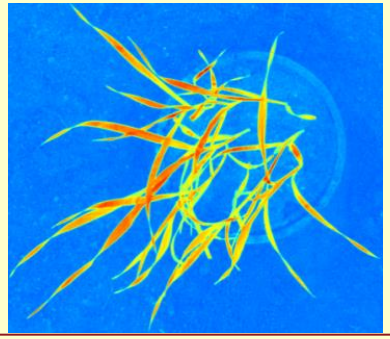


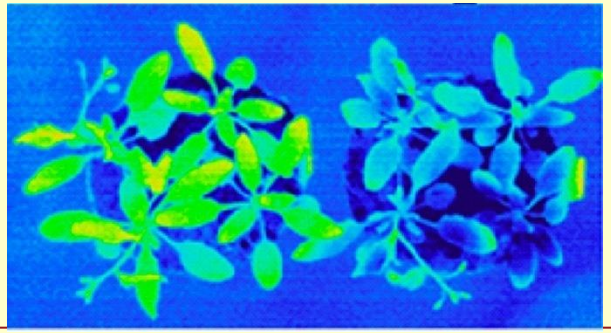


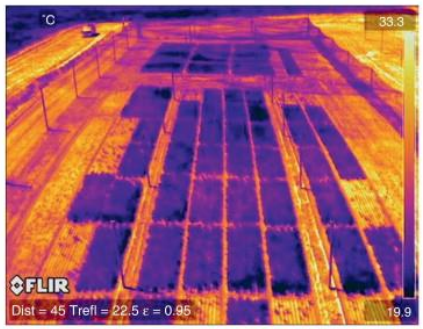


$$H = \frac{12}{n(n+1)} \sum_{i=1}^{k} \frac{R_i^2}{n_i} - 3(n+1)$$

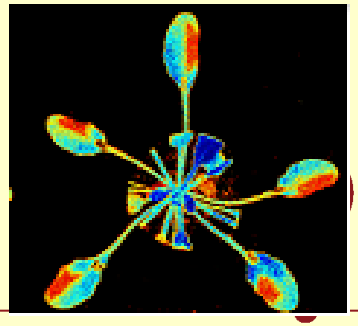
$$\text{recombination frequency} = \frac{\text{number of recombinant progeny}}{\text{total number of progeny}} \times 100\%$$



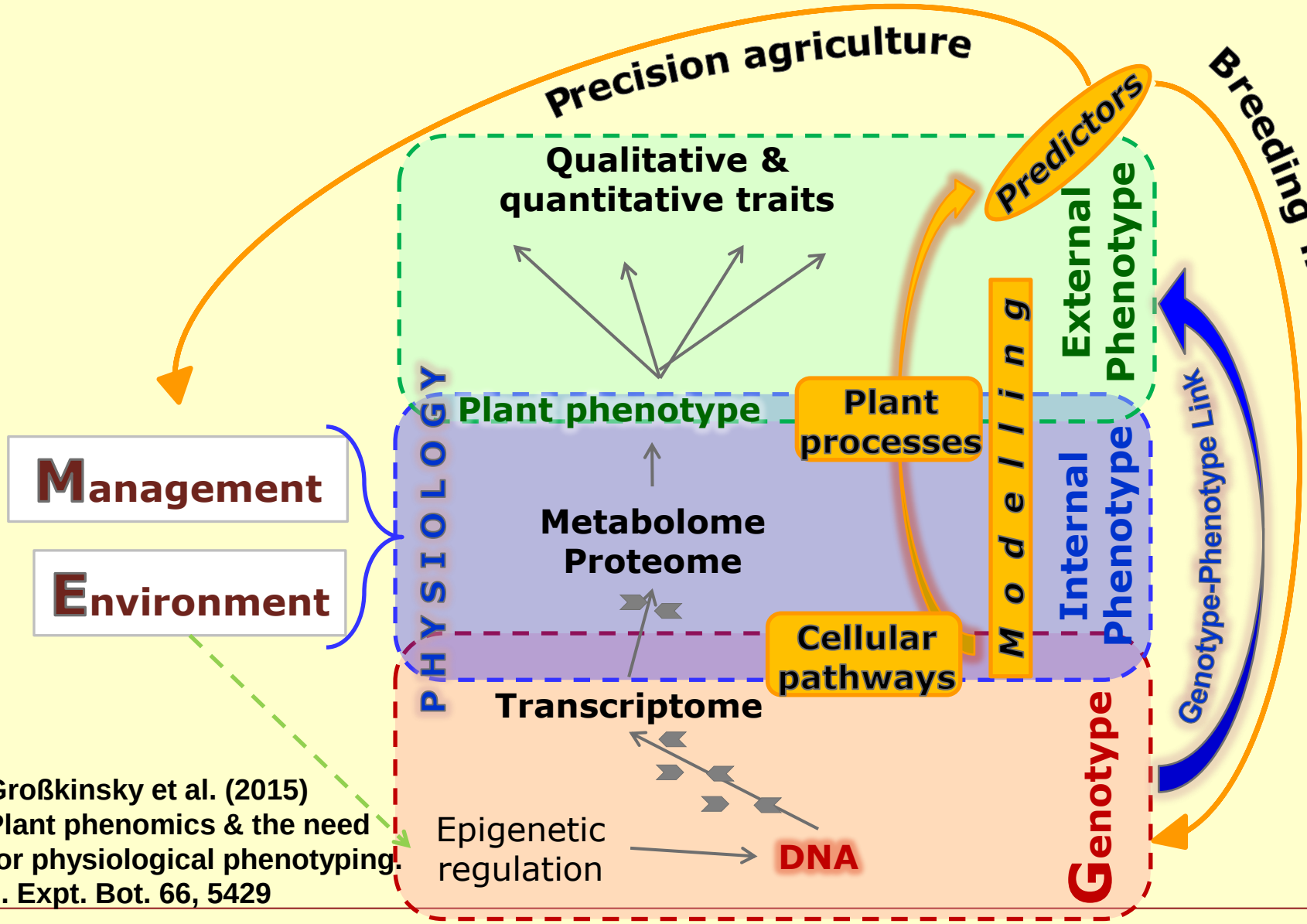




TRENDS in Plant Science

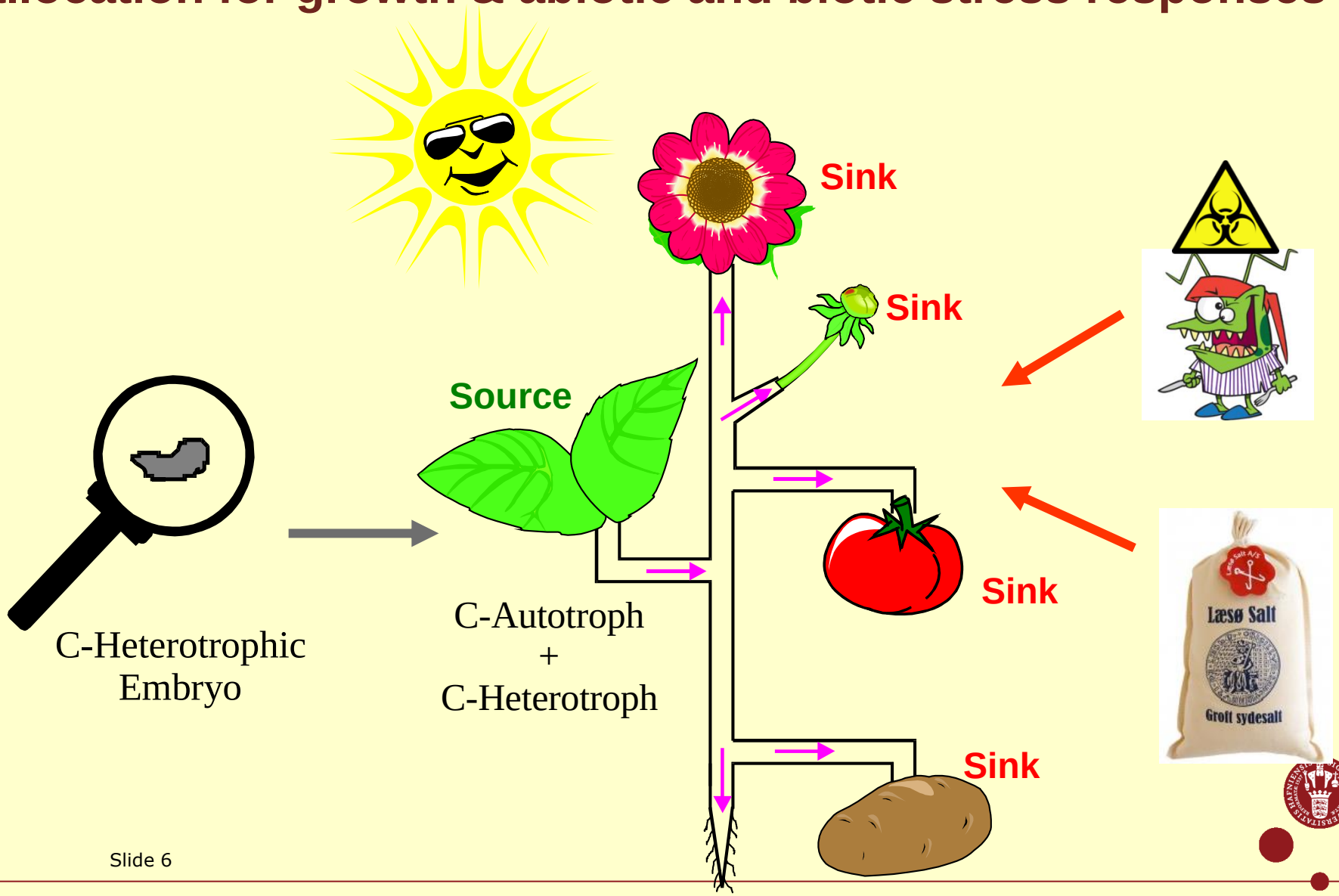


Plant Physiology – Key Interface between genome and quantitative traits that determines harvest yield and quality



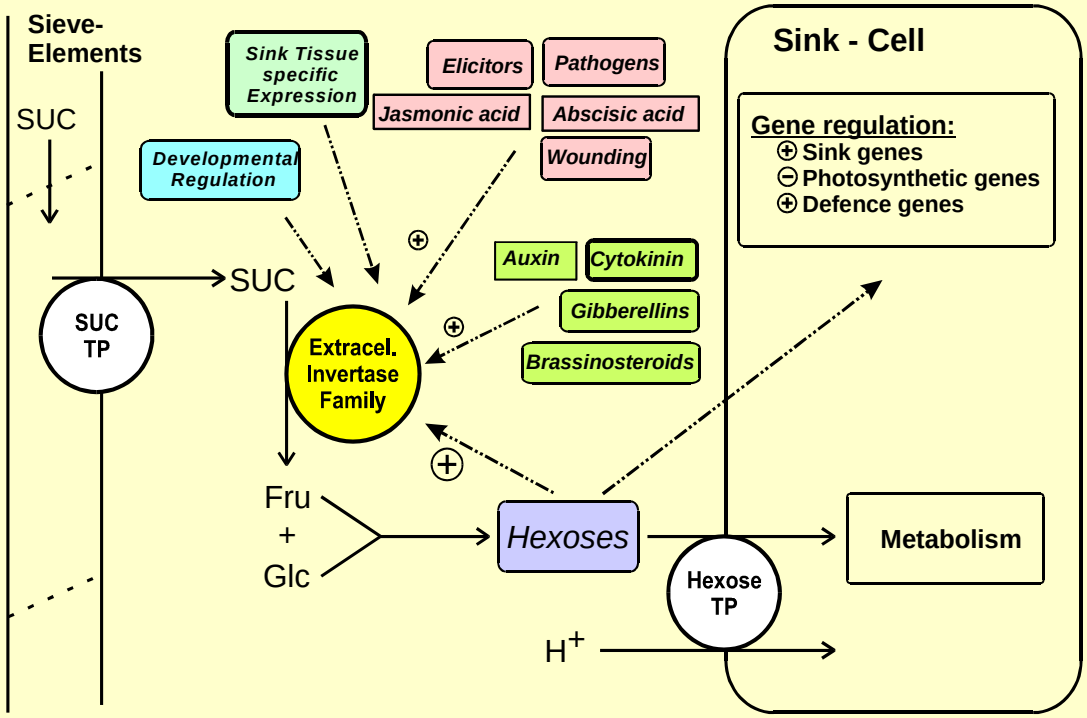
Großkinsky et al. (2015) Plant phenomics & the need for physiological phenotyping. J. Expt. Bot. 66, 5429

2. Central role of carbohydrate metabolism and resource allocation for growth & abiotic and biotic stress responses



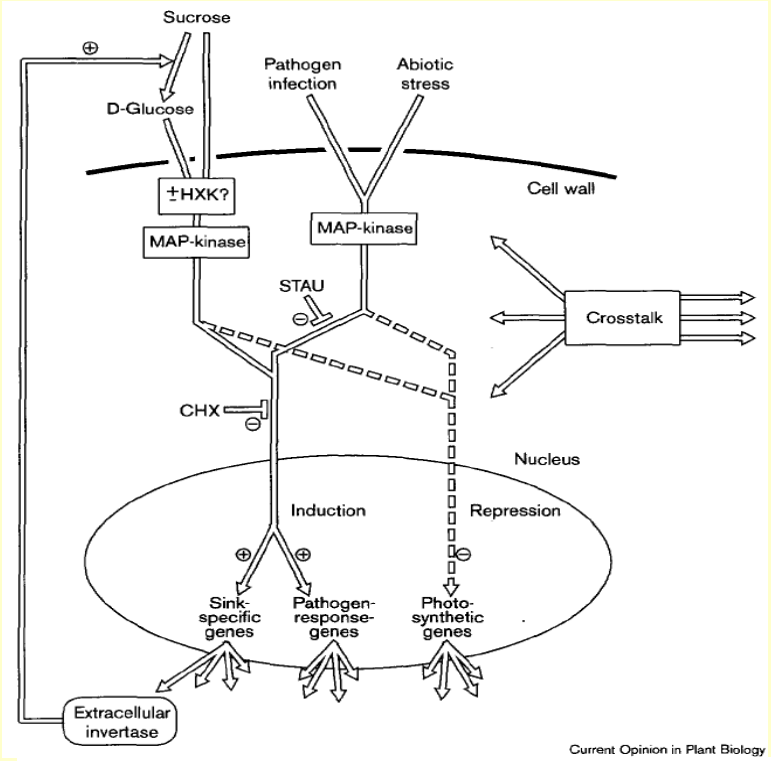
Development & stress responses are linked to plant carbohydrate metabolism

Extracellular Invertase: key metabolic & stress response enzyme



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

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



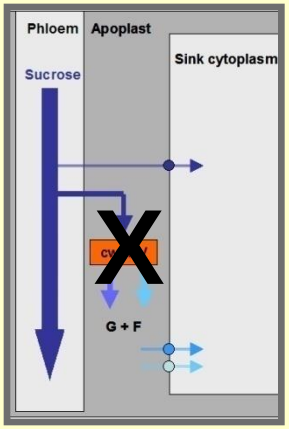
Roitsch (1999) COPS 2, 98
Berger et al. (2007) JXB 58, 4019
Albacete et al. (2011) Phyton 50,181

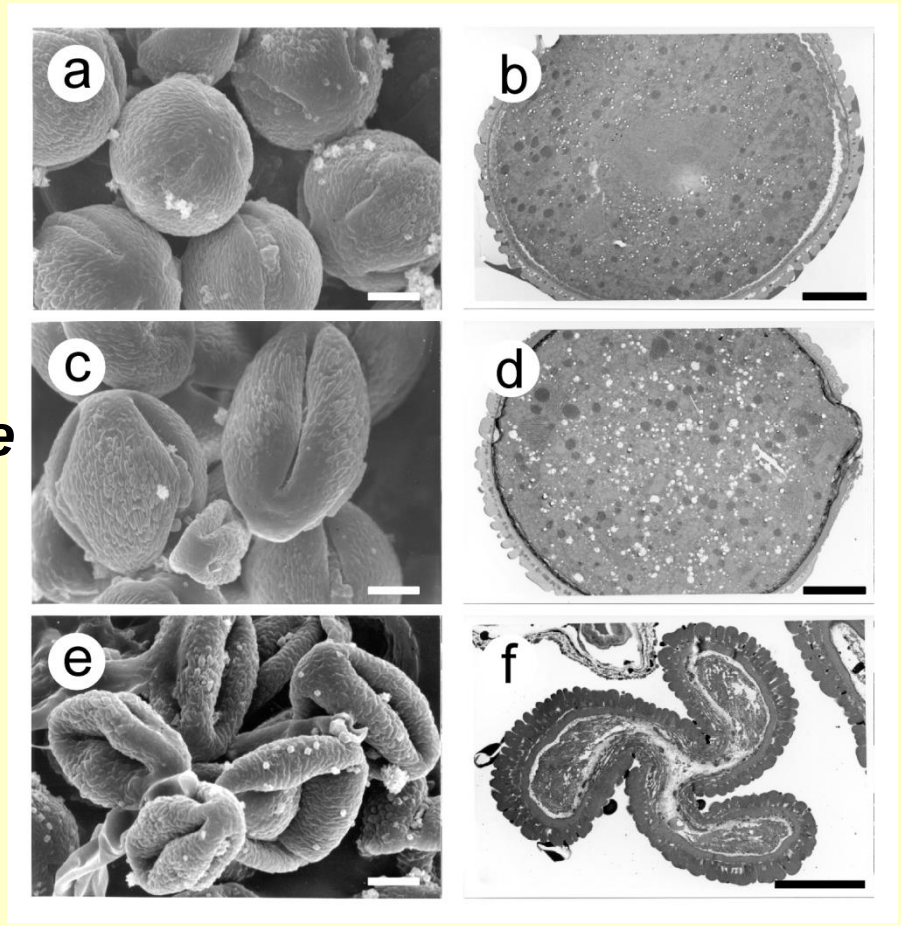
Extracellular invertase is essential for pollen development and reproductive stress resilience

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

SEM (2700x) TEM (5600x)

Nin88::
Nin88-Antisense





Wildtype

NT23-17

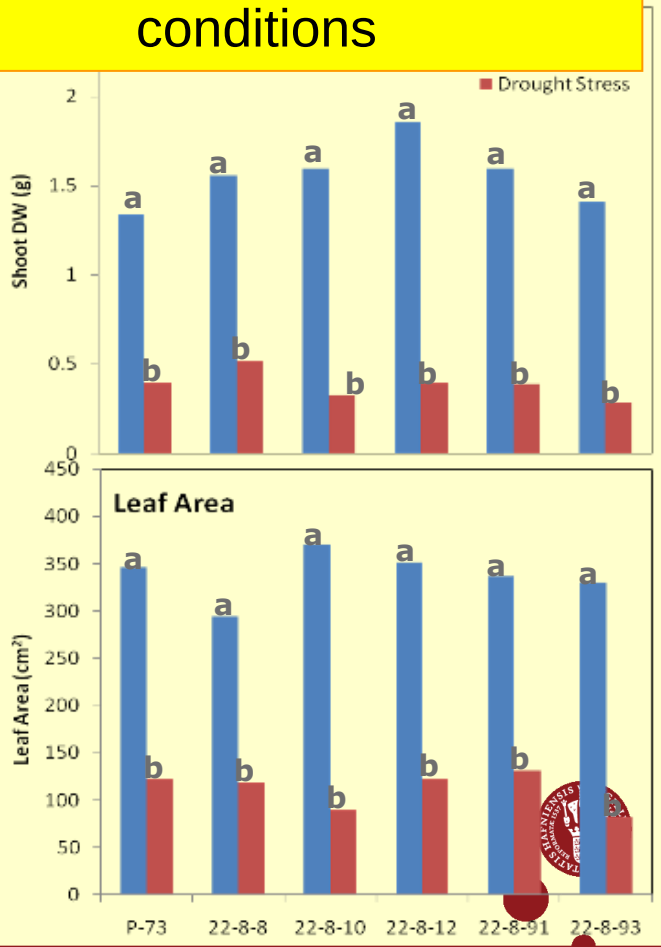
NT23-59

Goetz et al. (2001) PNAS
US Patent

Ectopic expression of extracellular invertase results in extreme drought stress tolerance

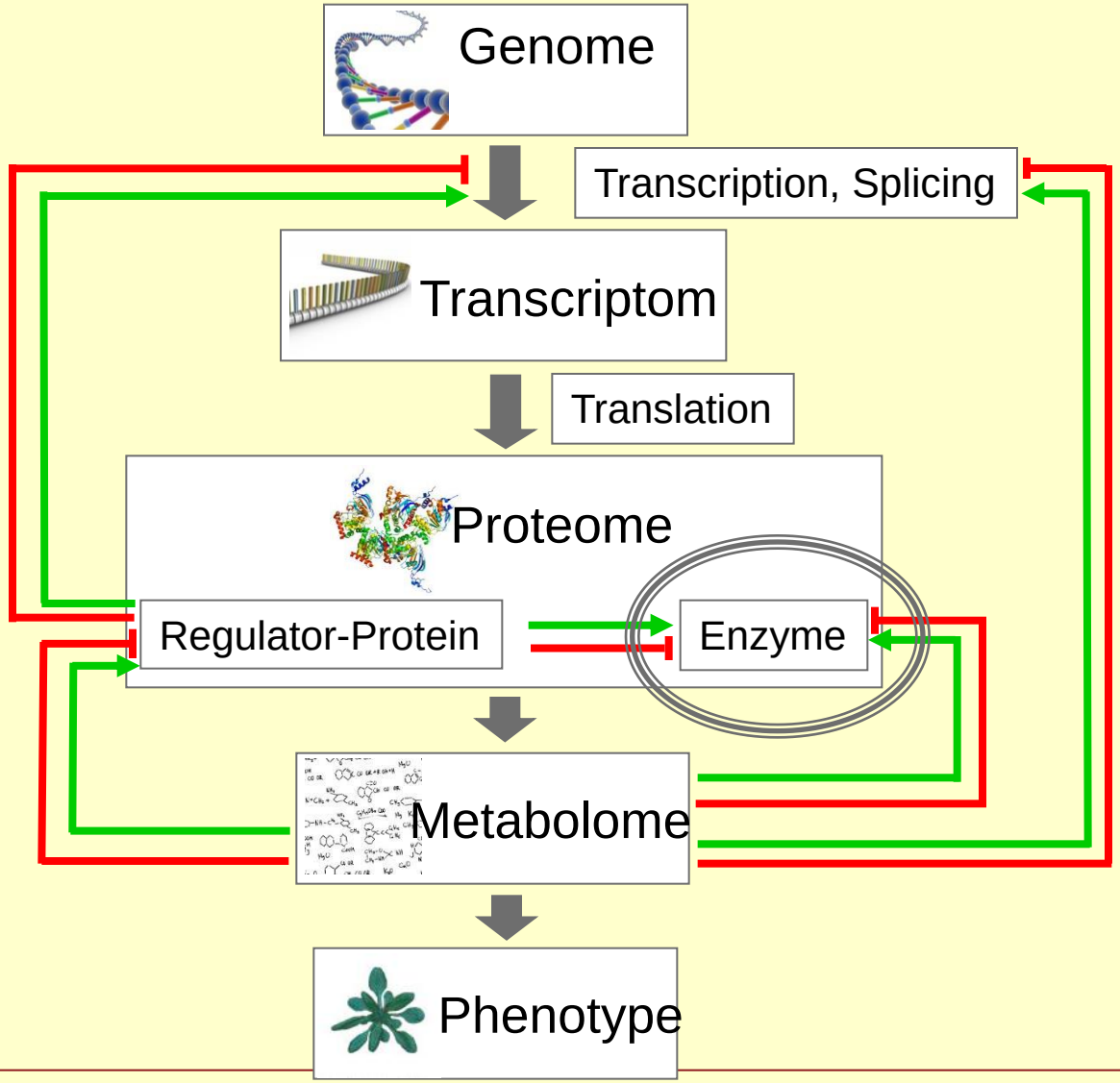
WT CIN1 1-91

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

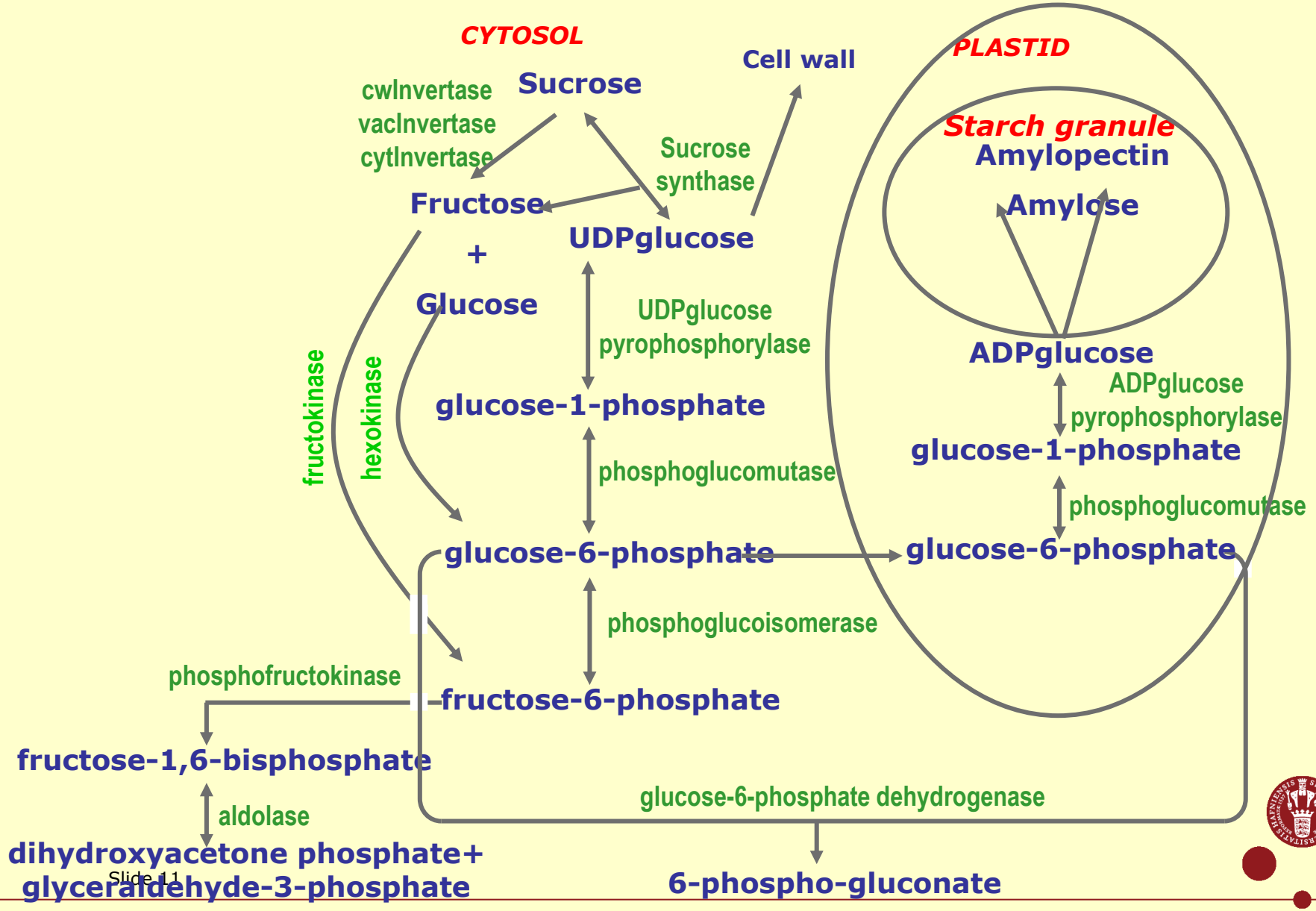


Albacete et al. (2014a/b) JXB 65, 6081 & 66: 863

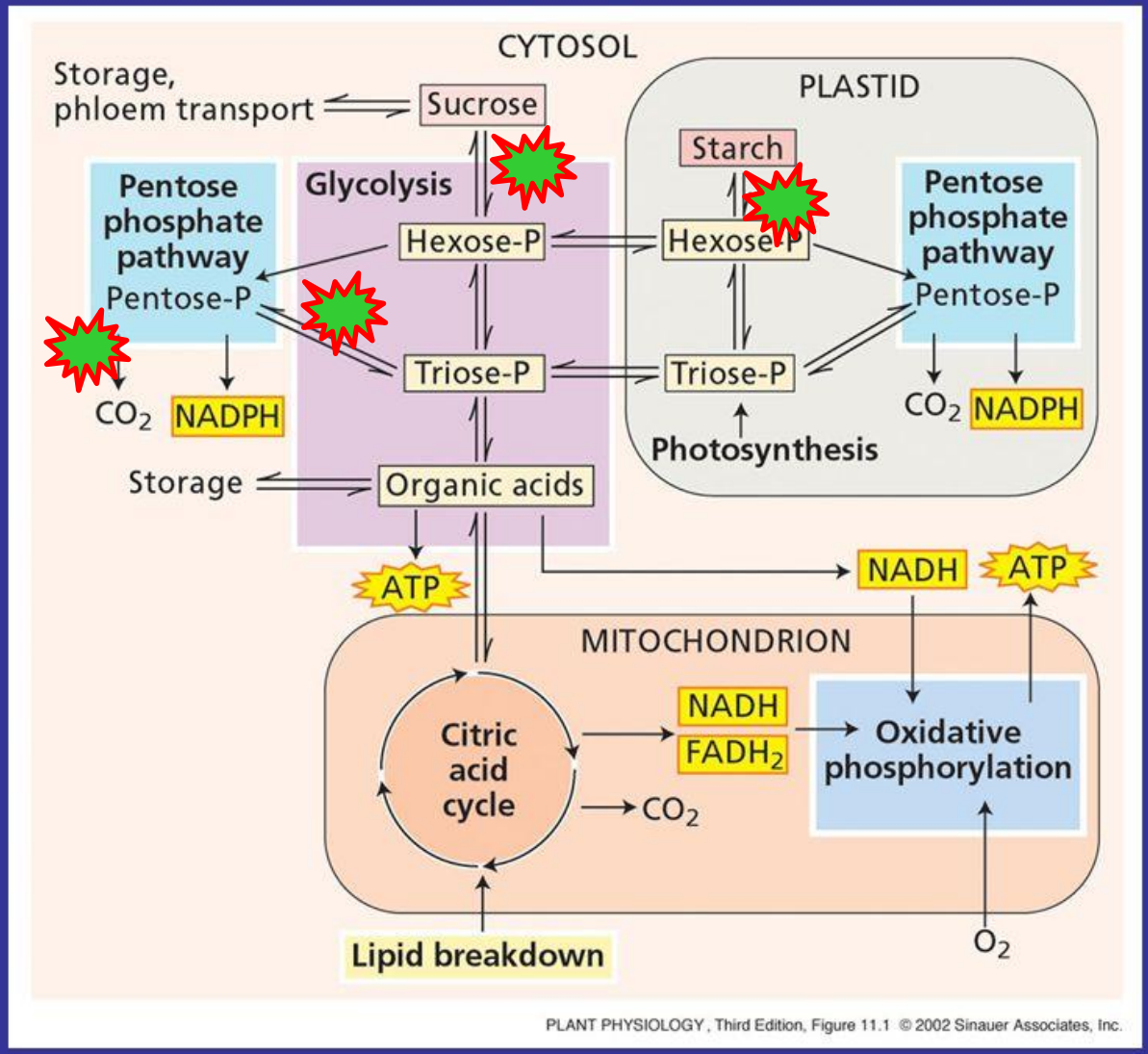
3. Physiological phenotyping of central carbon metabolism



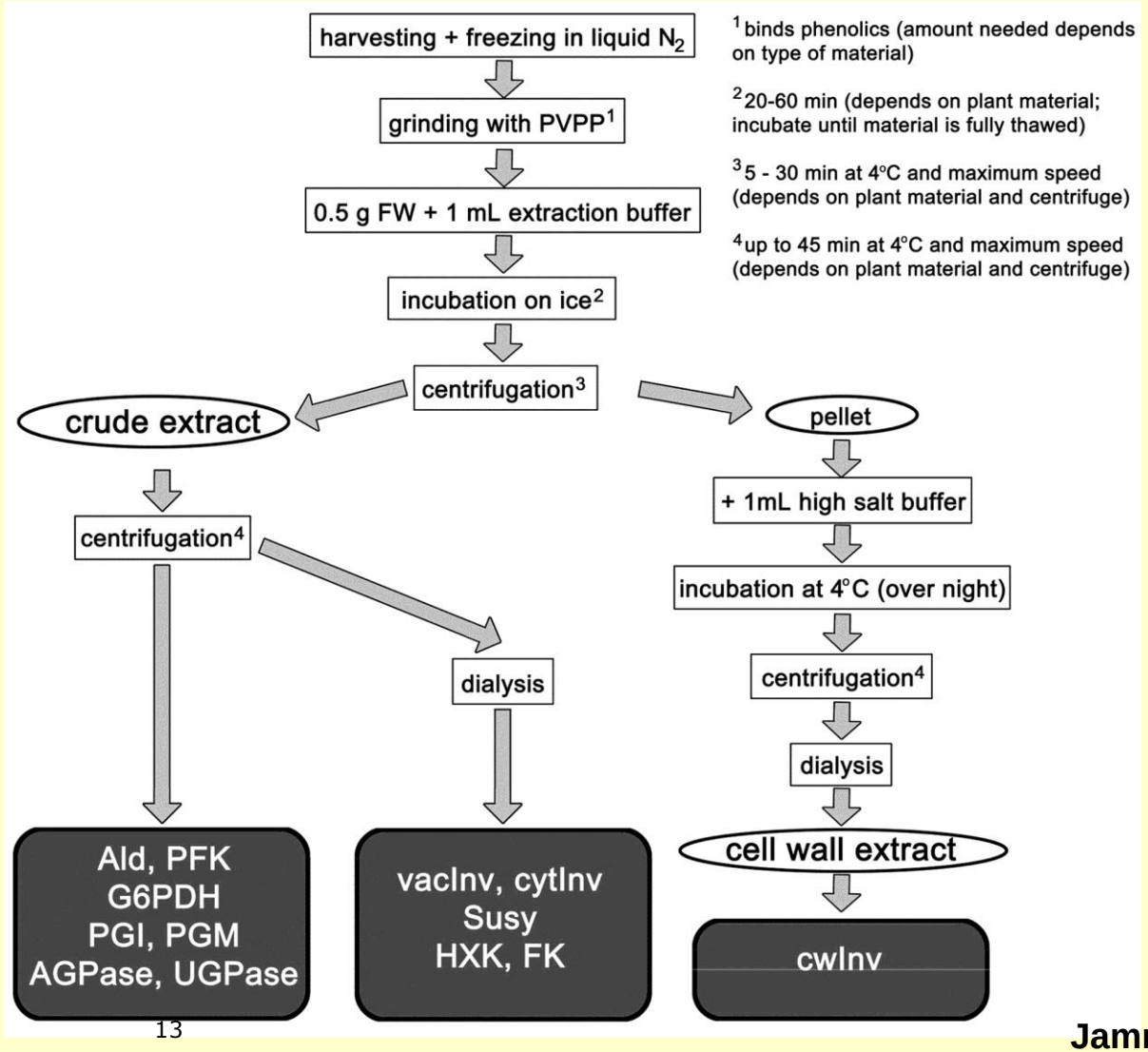
Carbohydrate enzyme activity signature



The enzyme activity signature covers key pathways of primary carbohydrate metabolism



Determination of complex enzyme activity signatures from one single extraction



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**

Jammer et al. (2015) JXB 66: 5531-5542

Examples of the identification of distinct enzyme activity signatures

Sugar beet: Negative correlation of biomass & sugar yield

- Distinct change of the enzyme activity signature during the transition from normal root to tap root development
- Identification of a metabolic switch between the main pathway of sucrose cleavage

Ryegrass: Determinants of the high sugar (fructan) trait

- Posttranscriptional, metabolic channelling into two pathways competing for the substrate sucrose in the vacuole
- Identification of a rate limiting glycolytic enzyme

Rapeseed: Nitrogen remobilisation during senescence

- Distinct enzyme activity signature of developmental and nitrogen starvation induced leaf senescence

Rapeseed: Impact of elevated CO_2

- Distinct enzyme activity signature under high CO_2

Wheat: impact of elevated CO_2

- Distinct enzyme activity signature under high CO_2

Barley: Reproductive heat stress tolerance

- Distinct impact of heat stress on the enzyme activity signature

4. Analyses of Robusta samples

4.1 Status sample analyses for 2/4 replicates

- 7 cultivars, 4 replicates at 5 locations

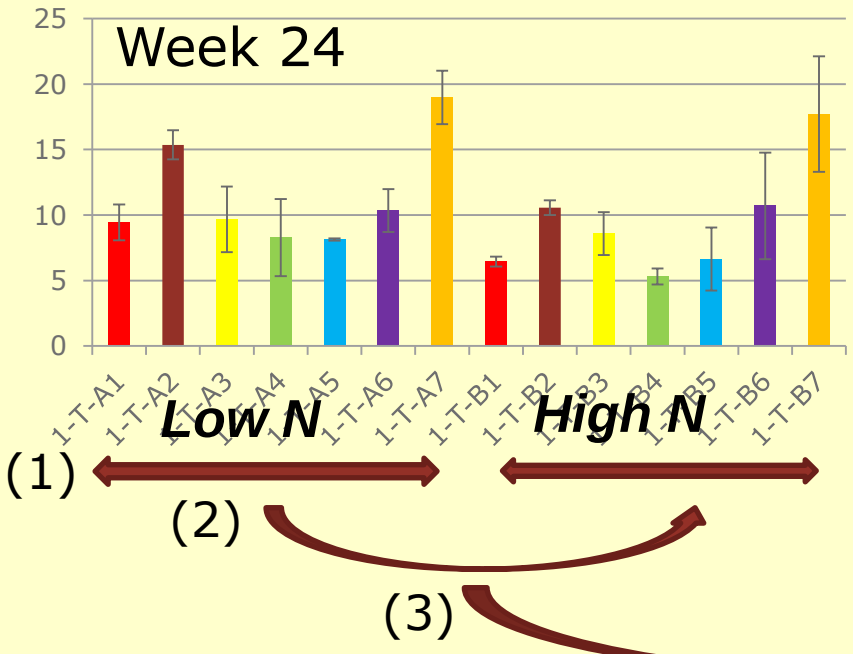
	Week 24 - flag leaf	Week 28 – flag leaf	Week 28 – grain
Taastrup N1 (100kg/ha)			
Taastrup N2 (140kg/ha)			
Ringsted N2			
Odense N2			
Esbjerg N2			
Holstebro N2			

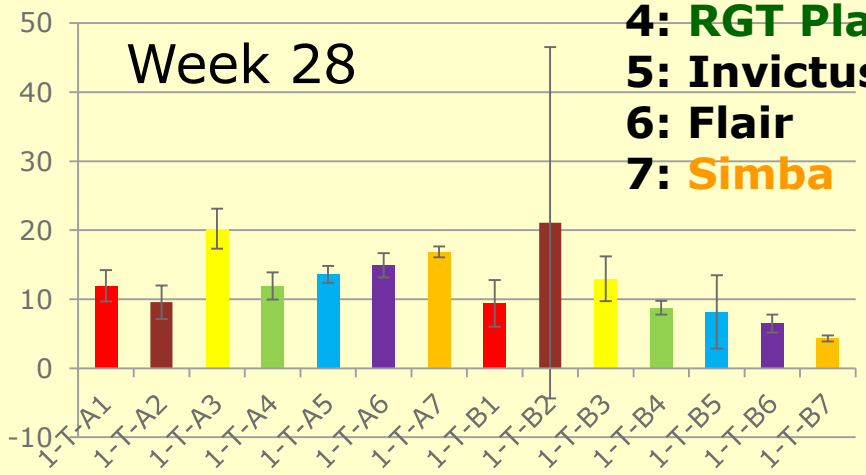
	Will not be analysed
	Analyses not yet started
	Assays in progress (2/4 reps, 13 enzymes)
	Data analyses (2/4 reps, 13 enzymes)
	Finished (2/4 reps, 13 enzymes)

4.2 Preliminary results: selected examples

- 7 cultivars, Taastrup, flag leaf
- Example: Enzyme "Aldolase"

1: Tocada
2: Laurikka
3: Evergreen
4: RGT Planet
5: Invictus
6: Flair
7: Simba





<u>Aldolase, and also other of the 13 tested enzymes, activity show:</u>

- (1) Large genotype specific differences
- (2) Nitrogen regime specific differences
- (3) Sampling time specific differences
- Different profile compared to some other enzymes
- Tissue specific differences (flag leaf vs. grain)?

Preliminary yield data

- ✓ Results represent some of the variation in NUE
- ✓ For %N the ranking was surprisingly constant among locations, Simba always highest , RGT Planet always lowest

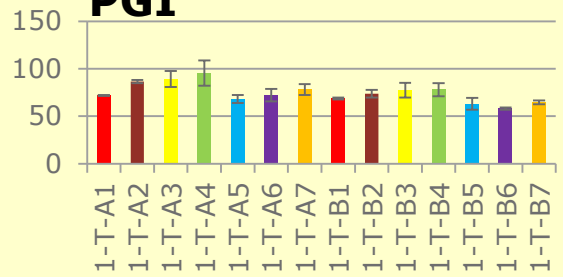
In progress:

Yield data from Taastrup (2 N regimes), biomass and N at flowering and biomass, N in straw and grain shortly before harvest

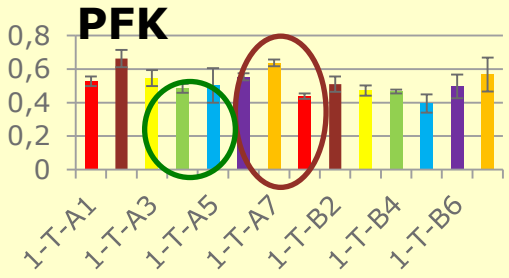
	DM (hkg/ha)	N yield (kg N/ha)	%N in grain
Tocada	45.1	83.8	1.87
Laurikka	49.4	90.5	1.85
Evergreen	49.7	89.5	1.81
RGT Planet	51.4	91.3	1.79
Invictus	49.2	89.0	1.82
Flair	48.7	89.7	1.86
Simba	46.5	91.3	1.98

Very preliminary (!) comparison of 2/3 Taastrup sample sets: enzyme activity vs. %N in grain

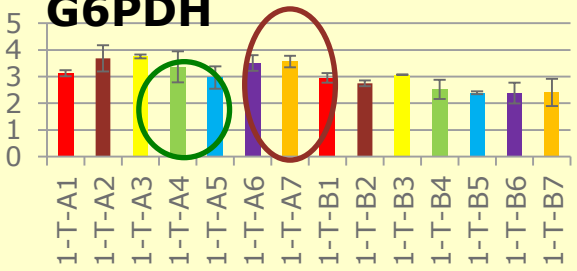
PGI



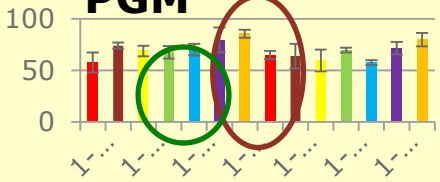
PFK



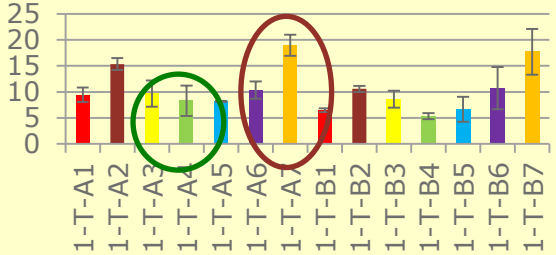
G6PDH



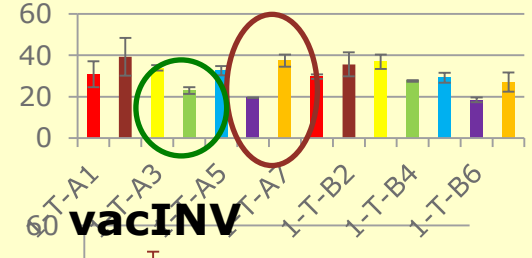
PGM



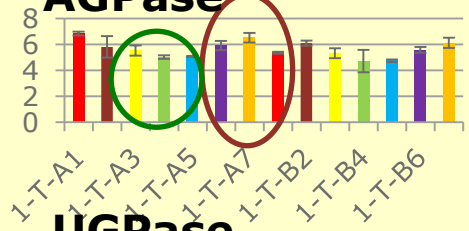
Aldolase



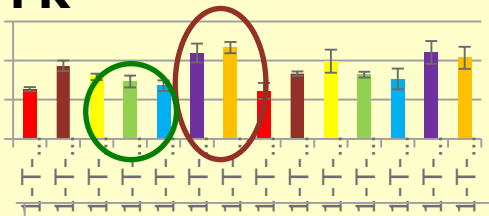
cwINV



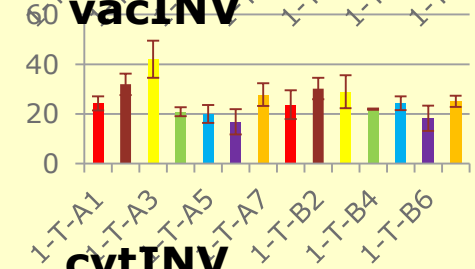
AGPase



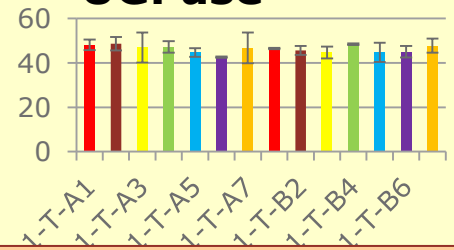
FK



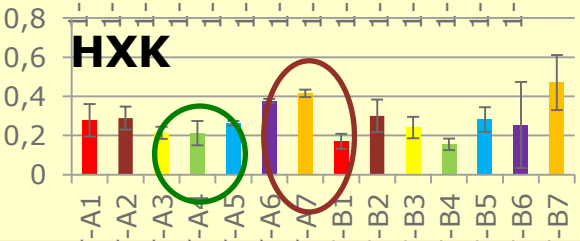
vacINV



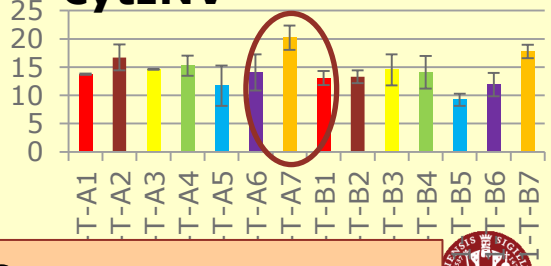
UGPase



HXK



cytINV

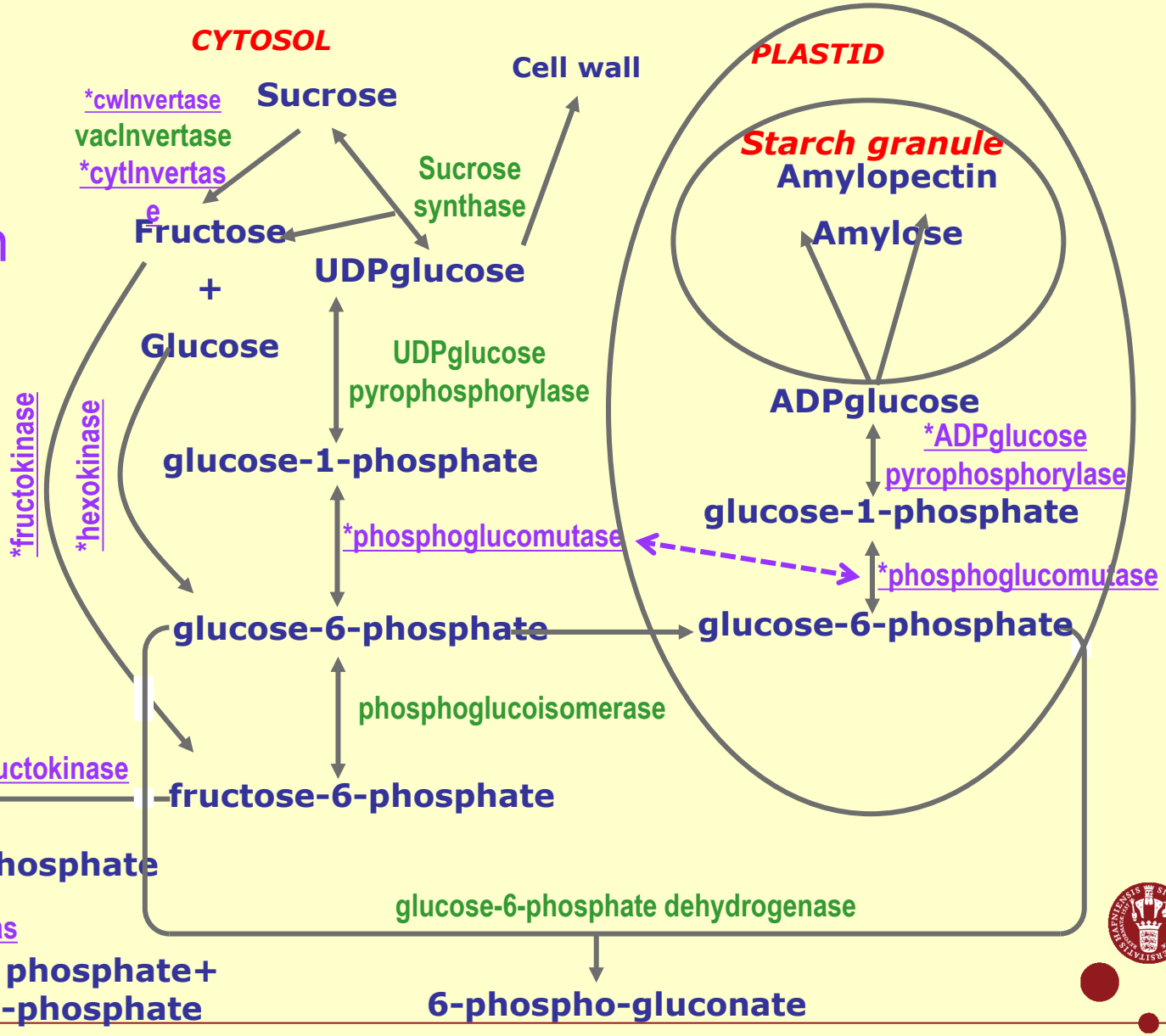


Simba, best in %N/grain (1,98%): among top 2 for 8/12 enzymes

RGT Planet, worst in %N/grain (1,79%): among last 2 for 7/12 enzymes

Carbohydrate enzyme activity signature

Simba vs RGT planet: High vs low -%N in grain -enzyme activities*



Further physiological analyses:

- Complete analyses for carbohydrate enzymes
- Establish and carry out measurements for nitrogen metabolism enzymes:
  glutamine synthase & nitrate reductase
- Eventually include also the determination of antioxidant enzyme activities as stress markers