

General problematic & challenge (talks C3_4-5-6)

- **Characterize traits controlling whole plant morphogenetic behavior**
(*'plant type', rice & sorghum vigor: e.g. organ size, appearance rate...*)
 - **Adaptive traits (abiotic stress) => phenotypic plasticity**
 - **Such a complex system relies on two key factors**
(*Fiorani et al. 2000; Heyer et al. 2004; Kobayazi et al. 2002; Liu et al. 2005; Roitsch et al. 2000*)
 - 1- **Meristem activity** (main site of decision) and its regulation
 - 2- **C metabolism (not only as a limiting factor)**
 - sugar signaling => meristem activity regulation
 - sugar based regulation of sink & source organ activity
 - **Even under drought:** not only hydraulics (C metabolism regulation/signal)
(*Foulkes et al. 2007; Liu et al. 2004, 2005; Roitsch et al. 2000; Trouverie et al. 2003.....*)
- ⇒ **Hypothesis: sugar based genotypic strategy => key adaptive traits?**
(*+/- C storage, +/- priority to sink organs*)

General problematic (2)

Meristem and C metabolism not easiest candidates for phenotyping

- Can modelling assist physiological analyses of such interactive processes?
=> Modelling – experiment feedbacks (test hypotheses...)
- Can modelling assist phenotyping?

Ecomeristem: formalize in a simple way these principles (vegetative phase)

- Many of the physiological concepts confirmed

(Fiorani *et al.* 2000; Heyer *et al.* 2004; Kobayazi *et al.* 2002; Liu *et al.* 2005; Roitsch *et al.* 2000)
(Dingkuhn *et al.* 2005, 2006, Luquet *et al.* 2006, 2007, Kim *et al.*...)

New steps:

- Addressing **water stress** (plant not C limited, but sink/source crucial)
- **Parameter connection to genetic information**
and genotypic physiological strategy

1 “Modelling drought effects on rice with EcoMeristem: Feedbacks of water and carbohydrate relations on phenotypic plasticity”.

2 Model assisted phenotyping of morphogenetic process diversity within sativa rice

3 Model assisted phenotyping of a rice mapping population for morphogenesis regulation related QTL detection: proof of concepts.

(15 min each)



Component 3

Modeling rice response to drought with EcoMeristem

Feedbacks of water and carbohydrate relations on phenotypic plasticity

CIRAD
D. Luquet
M. Dingkuhn
A. Clément-Vidal
N. Sonderegger

Material & methods: Ecomeristem existing concepts

Rice vegetative morphogenesis & phenotypic plasticity

Dingkuhn et al. 2006b Func PI Biol (32-4)

Luquet et al. 2006a Func PI Biol (32-4)

Meristem:
Organogenesis
under GXE control

Meristem: key site of genes controlling morphogenesis

=> Organ production (leave, tillers, roots: **plastochron**, **phyllochron**)

=> Organ dimensioning (**MGR**, Meristem Growth Rate)

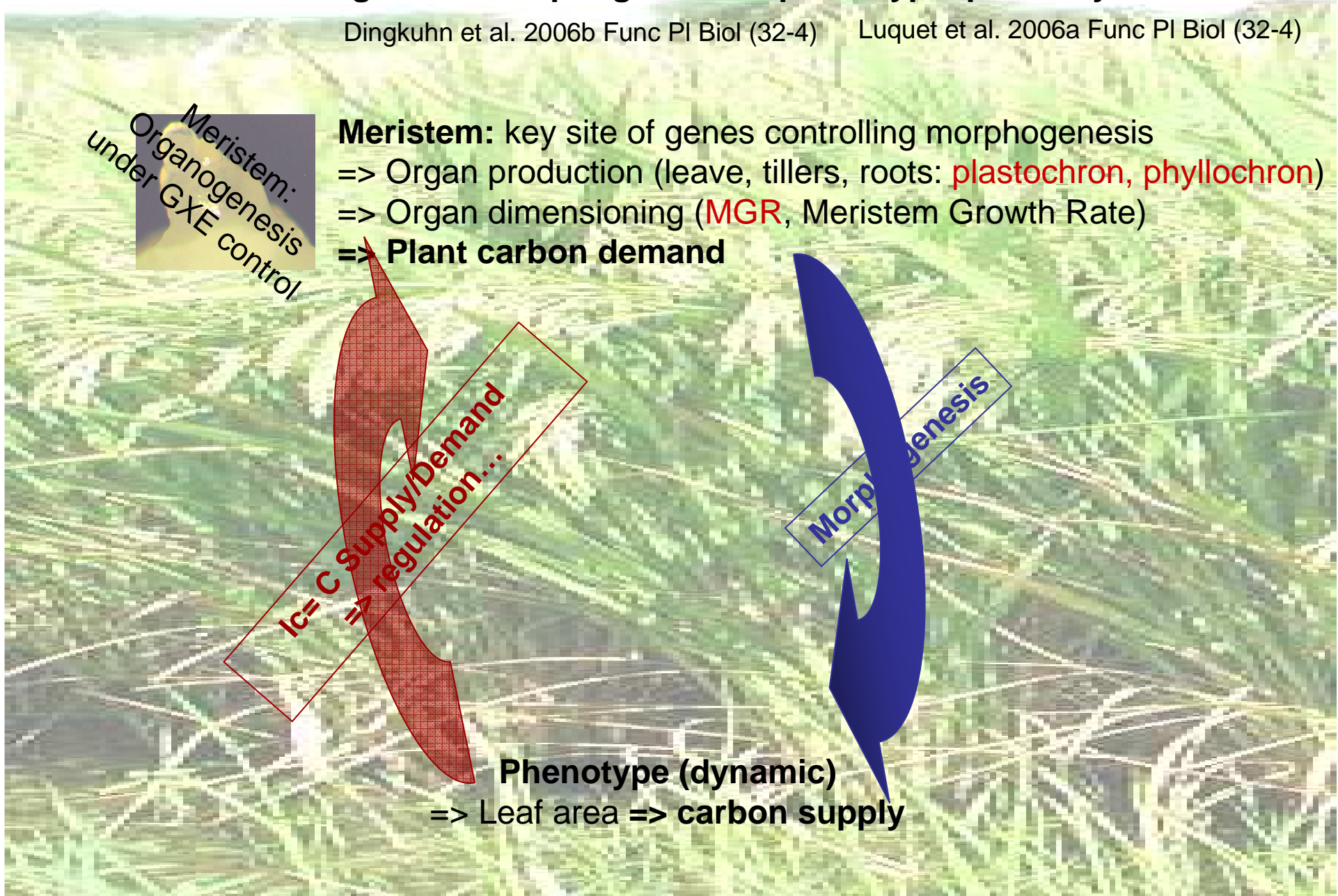
=> **Plant carbon demand**

**$I_c = C$ Supply/Demand
=> regulation...**

Morphogenesis

Phenotype (dynamic)

=> Leaf area => **carbon supply**



Ecomeristem :

Plant morphogenesis regulated by meristem response to plant internal competition for C lc (non limiting environment)

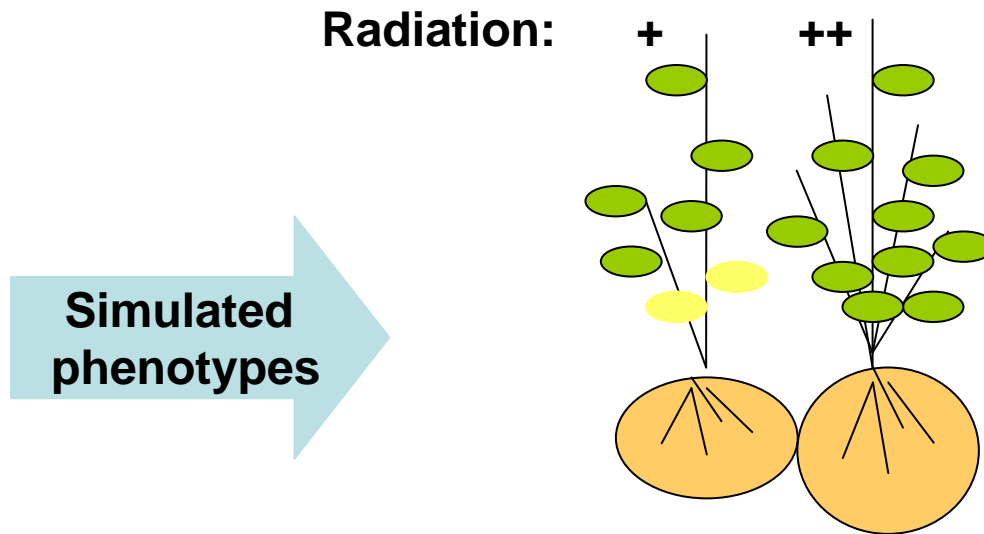
1) At each developmental step (*phyllochron*)

- If lc (C supply/demand) \geq genotypic threshold (ex: *lct* for tillering)
 - => tiller creation
 - => potential pre-dimensioning of new organs (**MGR**)
- else, organ pre-dimensioning regulated, no tillers

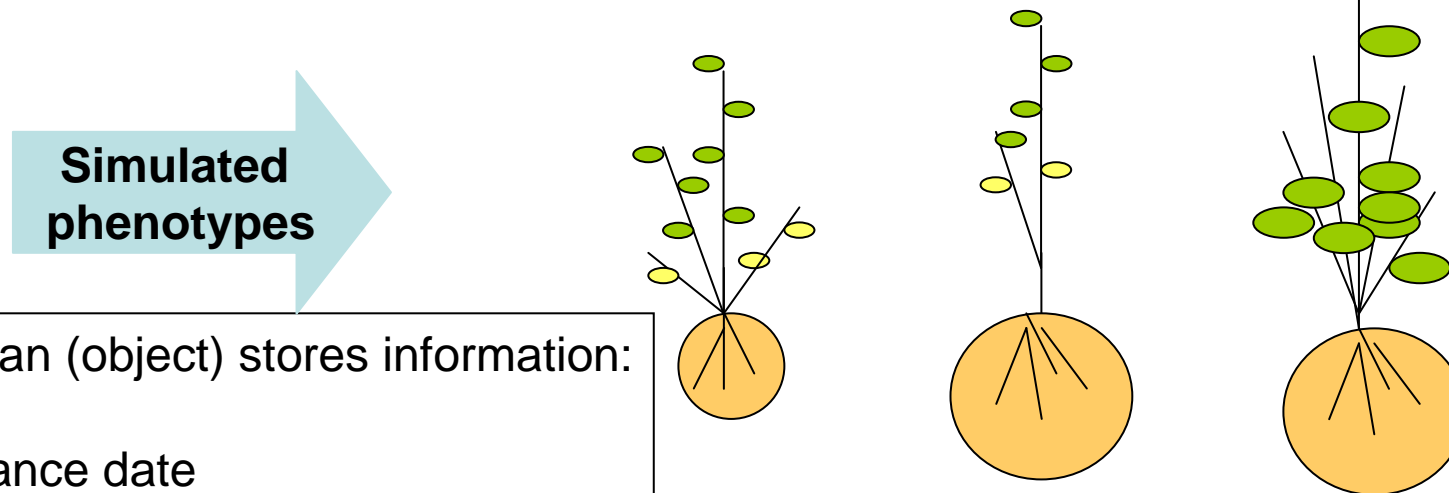
2) During organ expansion (growth):

- If $lc \geq 1$,
 - => C allocation to sink (expanding organs)
 - => C supply surplus stored (bulk reserve compartment)
- else, C reserve mobilized, growth slowed, senescence of oldest leaves

For a given set of parameters (phyllochron, MGR, Ict) ≠ radiation level:



For different sets of parameters in one environment:



Each organ (object) stores information:

- Rank
- appearance date
- Status (source, sink)
- Final or current dimension
- Expansion rate...

NB: root = one object...

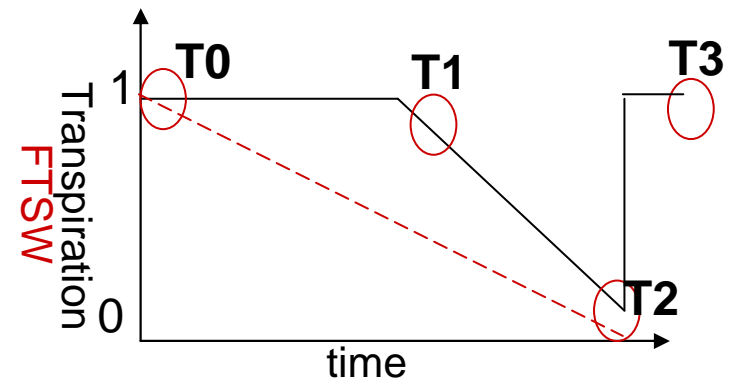
Material and methods (2)

- Drought impact on morphogenesis & metabolic source-sink processes
- Model implementation



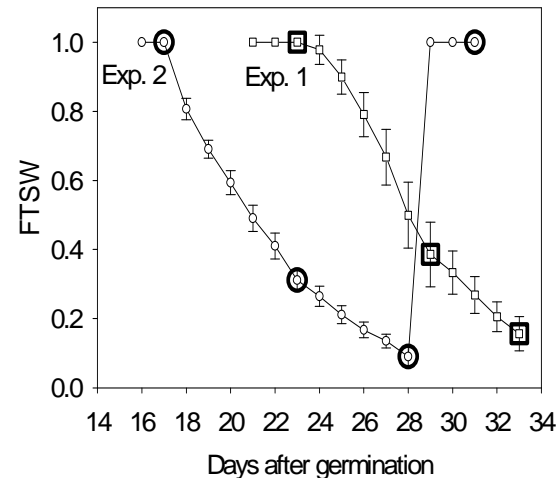
- 2 experiments (Exp1 & 2, 2006)
- Phytotron (~30d), **IR64**
- Stress applied at leaf 6-7 stage
- **Dry-down through plant transpiration**
- Pot weighed => transpiration vs. **FTSW**
($0 < \text{FTSW} < 1 = \text{AWC} / \text{TTW}$)

- Morphogenesis daily monitoring (leaf appearance, size, tillering)
- 4 sampling dates (mature & young, hidden leaves, roots)
 - dw, sugar content
 - invertase activity & gene expression (Exp1)
(related to organ sink strenght...)

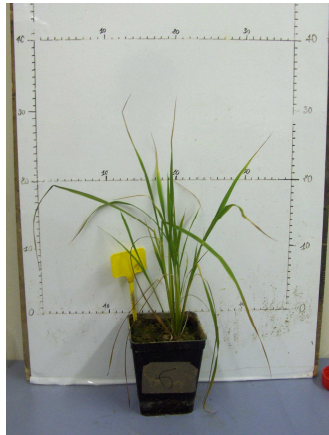


Experimental results

1- Stress quantification (FTSW)



2- Whole phenotype drought impact

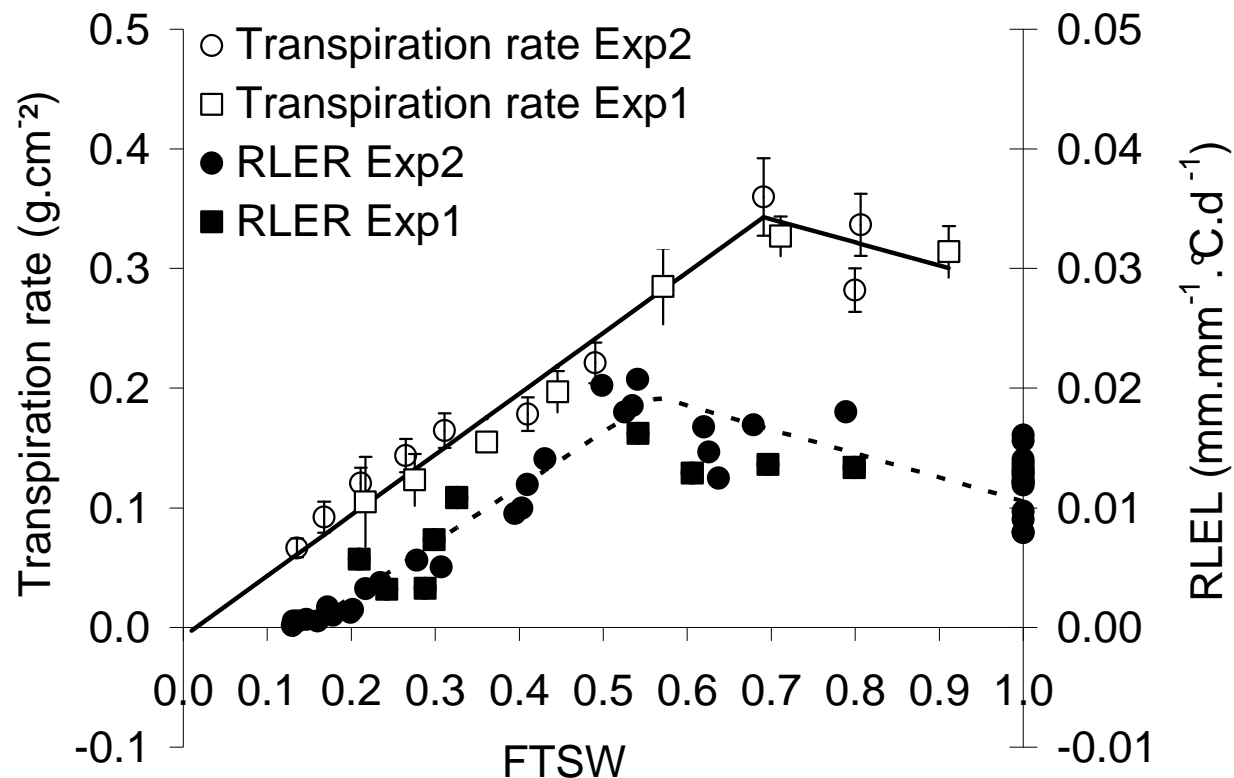


- 1 \searrow leaf transpiration
- 2 \searrow leaf expansion
- 3 \nearrow phyllchron
- 4 \searrow tillering
- 5 \searrow dw accumulation and LAI (\nearrow senescence)

- Which sensitivity of each trait to FTSW?
- Underlying metabolic pathways?



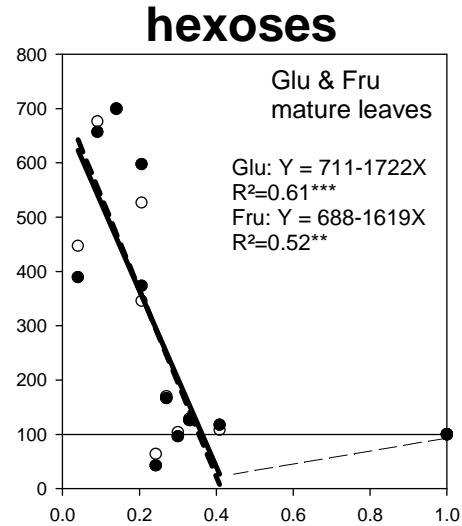
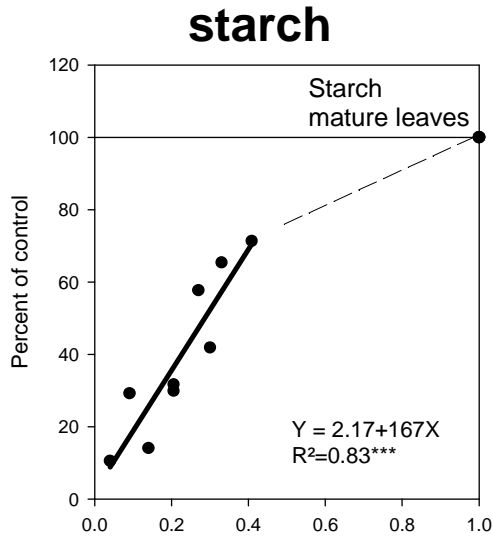
3- Leaf transpiration & expansion rates response to drought



- Robustness of results Exp1/Exp2
- Bi-phasic response (to be confirmed)?
- Threshold response lower for RLER (FTSW 0.56 vs. 0.69)
- Then more sensitive response of RLER (0 at FTSW 0.14!)

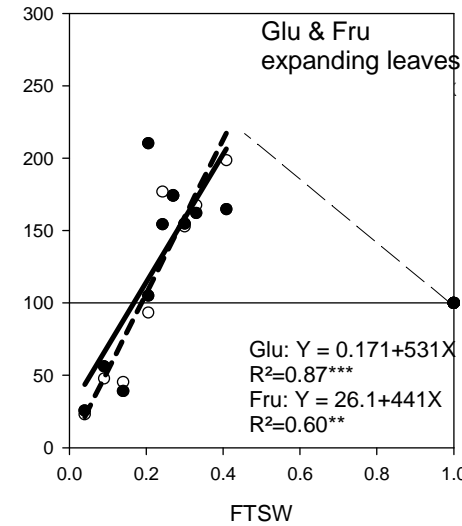
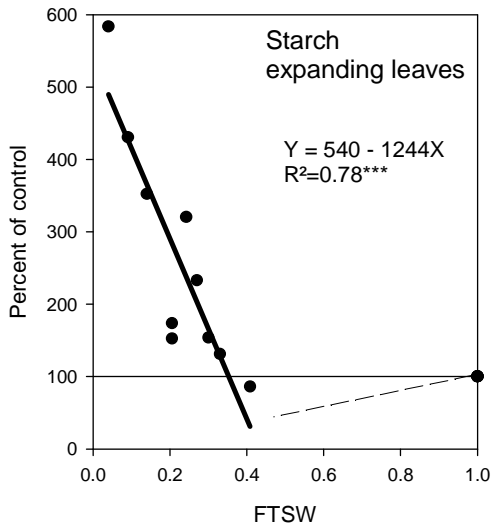


4- Sugar metabolism response to drought (Exp1 & 2)



Mature (source) leaves

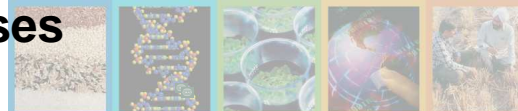
- Linear responses to FTSW...
- Opposition starch/hexose
- Opposition source/sink
- Bi-phasic? (~ RLER)
- Not large enough range of FTSW



Young (sink) leaves

starch

hexoses

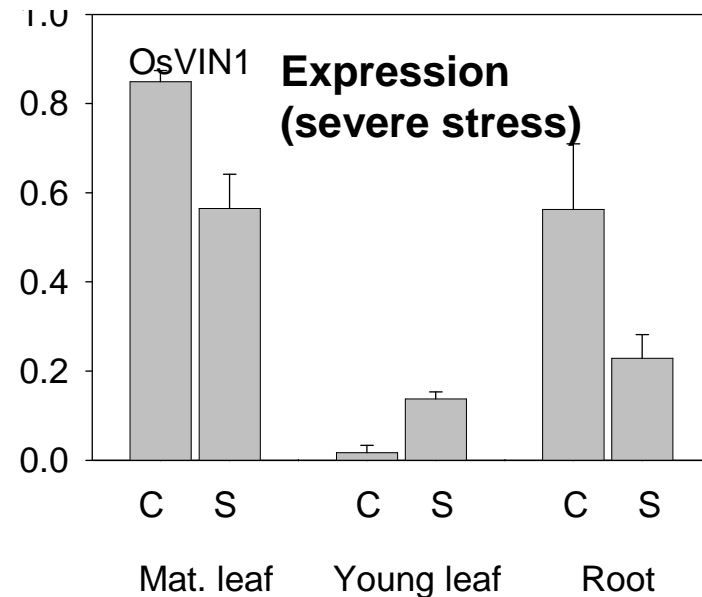
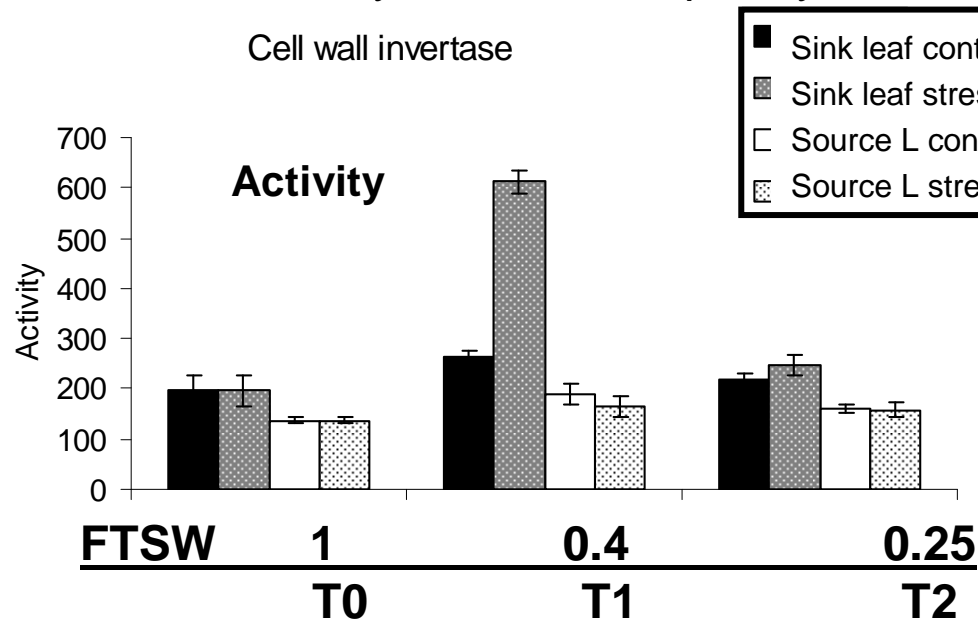


5- Relation with invertase activity & gene expression

Invertase => sucrose → glucose + fructose (hexoses)

Mild stress:

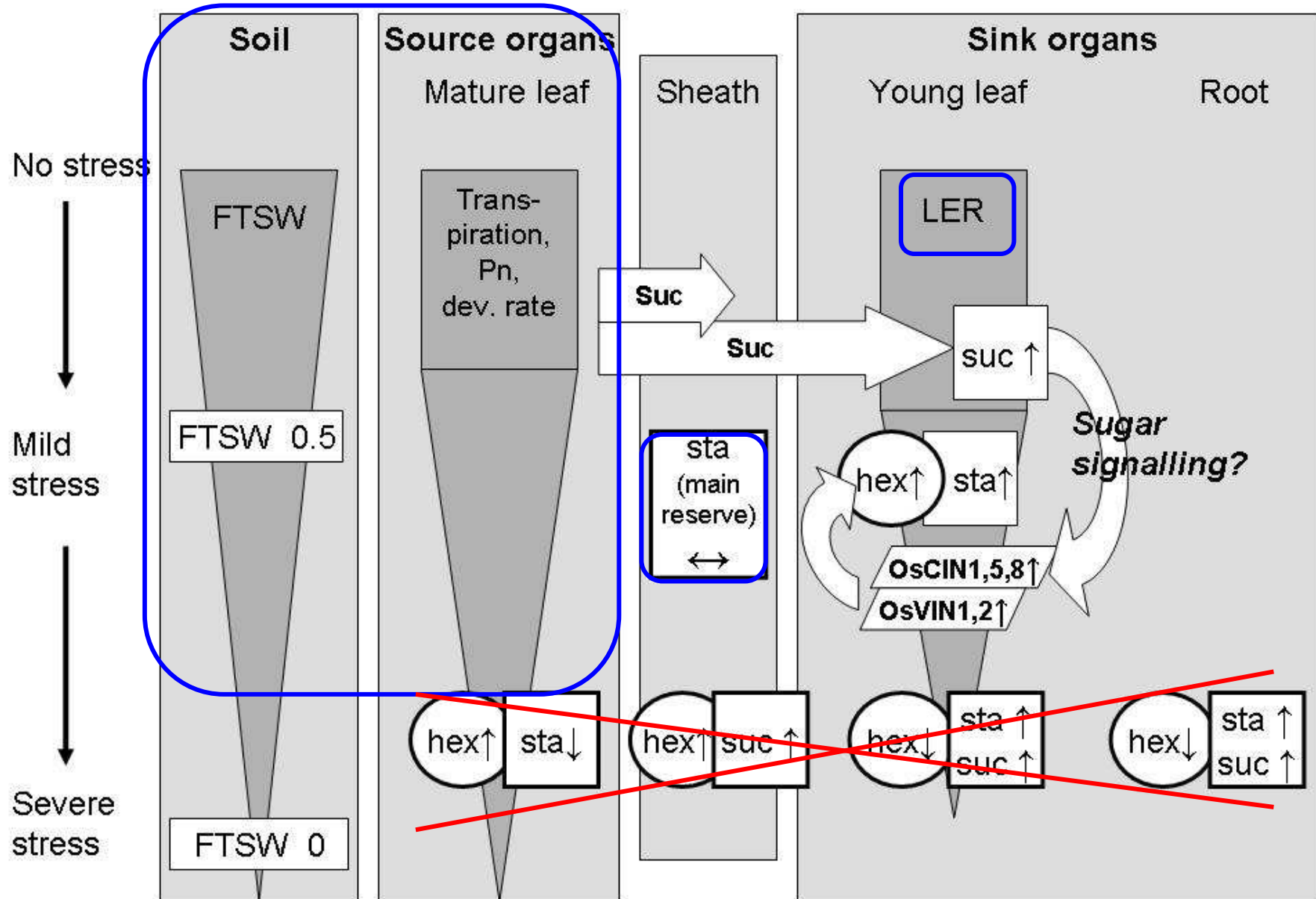
- invertase activity confirms temporary hexose increase in sink leaves



Severe stress:

- Most of invertase gene expression higher in sink leaves
- Not in agreement with hexose \searrow but could be explained by starch & sucrose \nearrow
- Starch & sucrose enzyme metabolism to be further explored
- Hexoses behavior 'switch' in sink leaves: which signal? hexose, sucrose?

Modelling concepts of rice plant morphogenesis response to drought



Model implementation

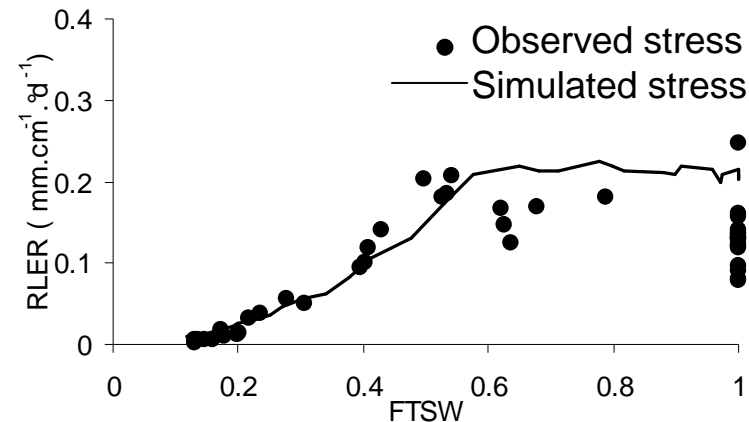
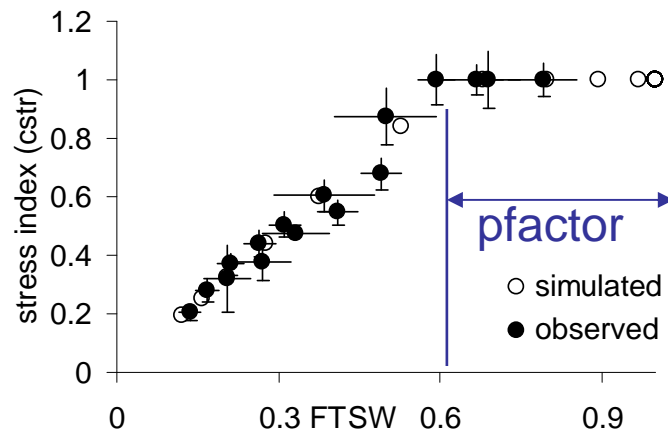
1- **Water balance** (separated transpiration & evaporation (LAI), soil property => **FTSW**)

2- **cstr**, water stress index = Tr_{act}/Tr_{pot} (actual by potential leaf transpiration rate)

$fn(FTSW, pfactor)$, **pfactor** genotype dependent

3- **cstr** impact **C assimilation** (source)

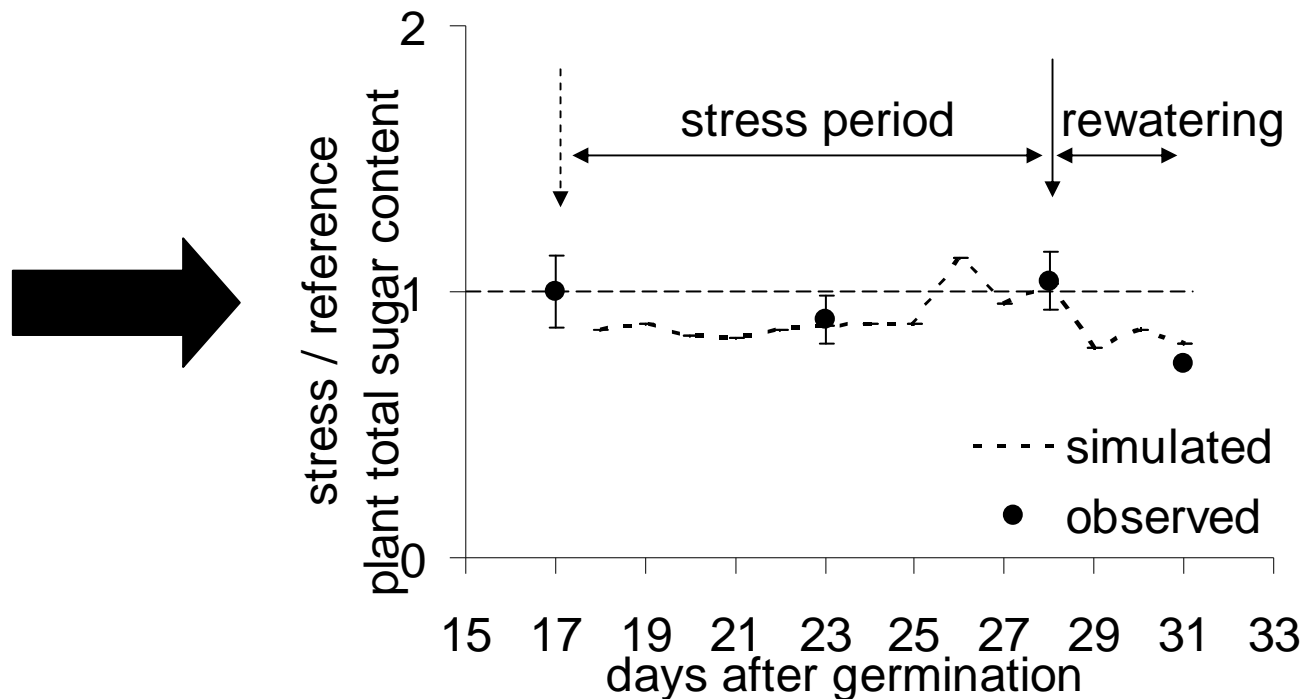
4- **FTSW** impact on **LER**



⇒ C sink ∨ (RLER => leaf appearance => tillering => plant dw & leaf area)

⇒ C source ∨ (assimilation rate, leaf area)

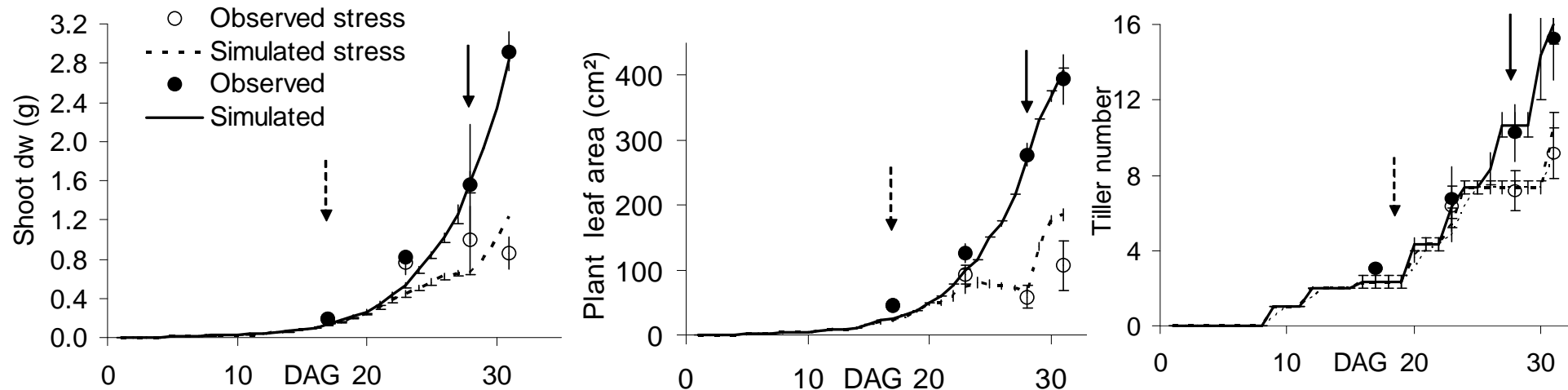
indirect regulation of whole plant C balance (sink to source adjustment)



- ⇒ Model accounts for non-limiting C (adjustment source / sink) under drought
- ⇒ No metabolic distinction between source & sink C storage or daily supply

Morphogenesis regulation (tillering, dw, LA)

1. Calibration (IR64)



calibrated		MGR	Ict	SLAp	Phyllochron
IR64	Average	1.19	0.98	38.18	37.12
	<i>SE</i>	<i>0.024</i>	<i>0.017</i>	<i>10.497</i>	<i>0.303</i>

⇒ Parameters optimized on control plants and used to simulate stress plants
 ⇒ stress response parameter provided by the experiment

Synthesis

- **Novel results on sugar source–sink** behavior in rice leaves under drought
- Opposite behavior starch / hexose in source & sink leaves
- (Biphasic) sugar response to FTSW to be neared from other processes
- Other enzymes must be addressed (starch & sucrose metabolism)
- Might be an interesting genotype based strategy to explore (cf. Azucena)

- **Model successfully implemented** (water stress)
- Key traits captured in the whole plant phenotype
- Rel. simple model of a complex system
- Same parameter values for Exp1 & Exp2 - control & stress plants
 - => Parameter values independent of treatment (unlike study on P stress)

Synthesis (2)

- **Process response to FTSW => phenotype & C balance well simulated**
- **New parameters: e.g. pfactor, LER response to FTSW**
- => Questions on optimization methodology...**
- **A way to indirectly phenotype sink to source regulation?**

- **Model to be tested on other genotypes under drought**
- **2008: detailed drought study on 12 rice genotypes**
- **Necessary step before model application for larger scale phenotyping**
- **First step (no stress):** next presentations for medium throughput application



Component 3

Model assisted phenotyping of morphogenetic process diversity within sativa rices

CIRAD

D. Luquet

A. Clément-Vidal

M. Dingkuhn

IRRI

K. Mc Nally

T. Lafarge (CIRAD – IRRI)

Objective

**Added value of model parameters as traits for
genotype discrimination & genetic studies?**

Material and methods

- 201 sativa rice genotypes (core collection GCP, ADOC)
 - Vegetative morphogenesis, rainfed conditions (IRRI, April 06),
 - 3 blocks & 2 treatments = 2 samplings
 - S1 (35 DAE): reference sampling
 - S2 (55 DAE): sampling after water stress (soil dry down from S1)
 - BUT typhoon 3 days before S2 => data too complex to analyze...
 - Sampling on 1 plant per block:
 - Tiller & main stem leaf n°, last ligulated leaf rank, size, dry weight (dw)
 - Main stem sheath for sugar analysis
 - Shoot dw
- + Germination test for model initialization (grain dw, leaf1 size, root/shoot dw)

⇒ 3 categories of traits:

- Measured conventional traits
- Model process based parameters optimized on 201 x 3 target files (measured)
- Sugar content analysis



Genotypes

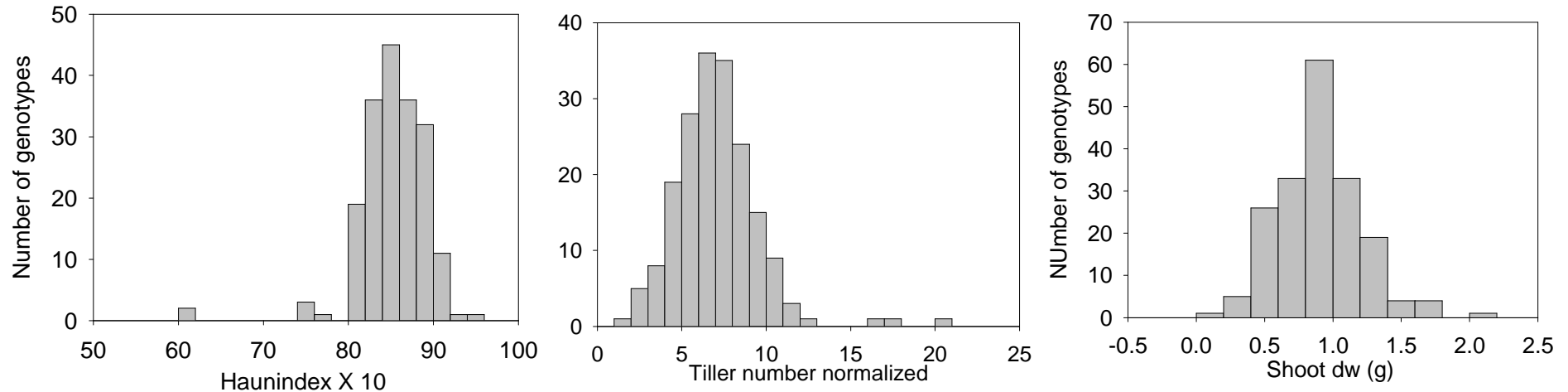
- 3 reference genotypes: Apo, Vandana, IR64 (doubled in the experiment)
 - 201 genotypes within 7 genetic groups

Genetic group	Genotype number
1- Japonica tropical	23
2- Indica	135
3- Japonica temperate	10
4- Aromatic	17
5- Aus/Boro	11
6- "Intermediate"	2
7- Deep water	4

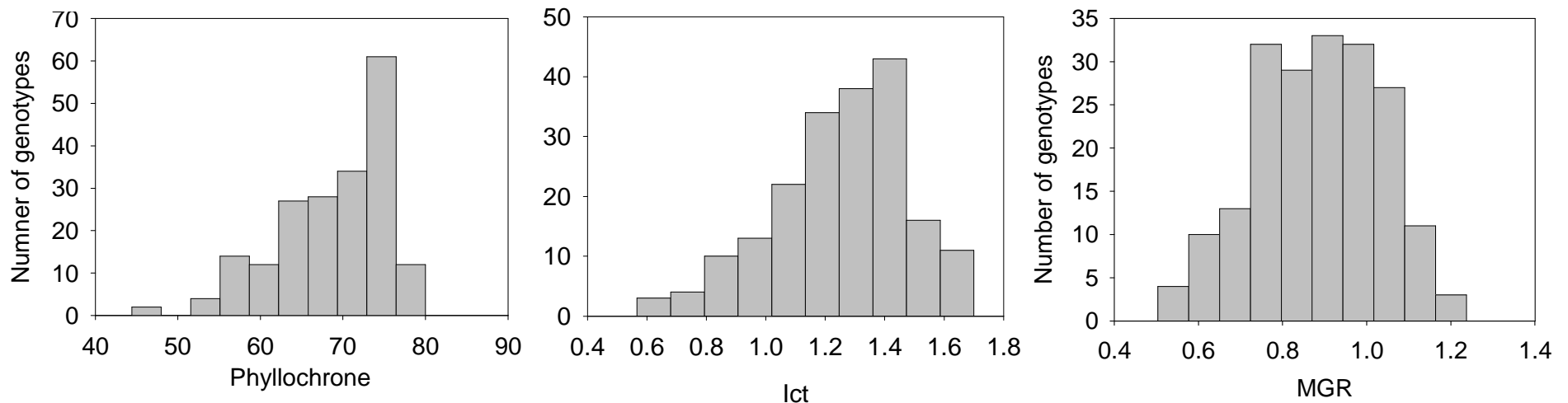
⇒ Discriminative capacity of process based vs. complex traits among genotypes & genetic groups?

Results - variable distribution at S1

- **Measured variables (classical traits)**



- **Related optimized model parameters**

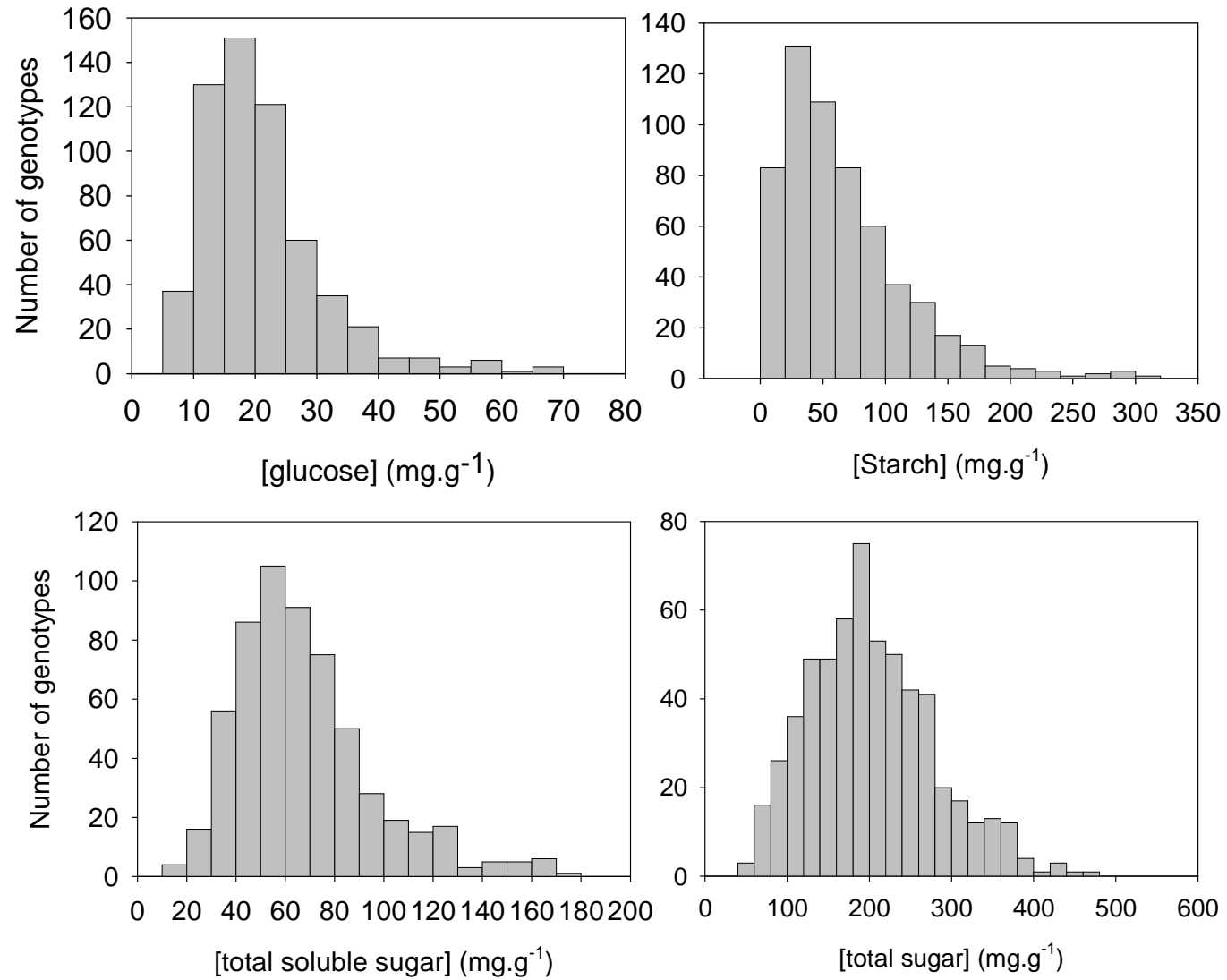


All variables show a normal distribution



Generation Challenge Programme
CULTIVATING PLANT DIVERSITY FOR THE RESOURCE POOR

- **Biochemical variables**



All variables have a normal distribution

Results - ANOVA

Type	Variable name	Line effect	Genetic group effect
Observed	Tiller nb	Y	Y
Observed	Shoot dw	Y	Y
Observed	Haun index	N	N
Observed	LLL / rank	Y	Y
model	MGR	Y	Y
model	Phyllo	N	N
model	Ict	Y	Y
model	SLAp	N	N
Biochem	Glucose	N	N
Biochem	sucrose	N	Y
Biochem	starch	Y	Y

Each trait category with at least one with genotype and/or genetic group

Results – regression analysis (shoot dw)

	LLL	LLw	TN	haun inde:	MGR	Ict	SLAp	Phyllo	R ² adjusted
1 variables						X			0.41
2 variables	X		X						0.60
3 variables	X	X	X						0.70
4 variables	X	X	X					X	0.75
5 variables	X	X	X	X				X	0.76
6 variables	X	X	X	X		X		X	0.77
7 variables	X	X	X	X	X	X		X	0.77
8 variables	X	X	X	X	X	X	X	X	0.77

- ⇒ Ict (tillering) parameter is more explicative than measured tiller number (TN)
- ⇒ Phyllo parameter better than corresponding variable (haun index)
- ⇒ Leaf size (LLL & LLw) is not substituted by any parameter

Correlation between sugars & others traits

	Glucose	Fructose	Sucrose	Soluble sugars	Starch	Total sugars
LLL	0.02	0.07	0.10	0.09	-0.32	-0.21
LLLw	0.04	0.07	0.13	0.12	-0.12	-0.03
TN	0.02	0.01	0.05	0.04	-0.10	-0.06
SDW	0.06	0.08	0.21	0.17	-0.18	-0.04
Haun index	0.00	0.01	0.20	0.12	-0.15	-0.05
First leaf length	-0.09	-0.10	0.02	-0.07	-0.09	-0.12
Seed weight	-0.10	-0.12	-0.09	-0.14	-0.13	-0.20
RDW/SDW initial	0.06	0.06	-0.05	0.02	0.10	0.10
MGR	0.03	0.11	0.10	0.11	-0.14	-0.04
Ict	-0.08	-0.09	-0.15	-0.15	0.15	0.03
SLAp	0.03	0.02	-0.09	-0.03	-0.02	-0.03
Phyllo	-0.04	-0.04	-0.18	-0.13	0.04	-0.05

1- Sucrose & starch show significant correlation with SDW

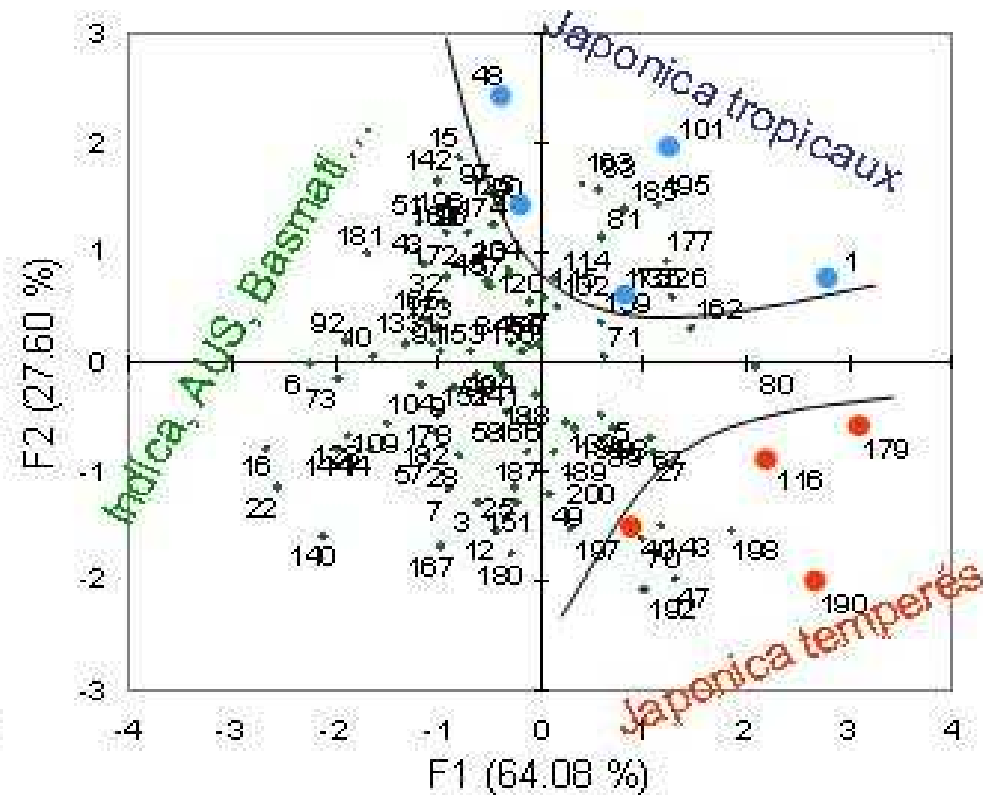
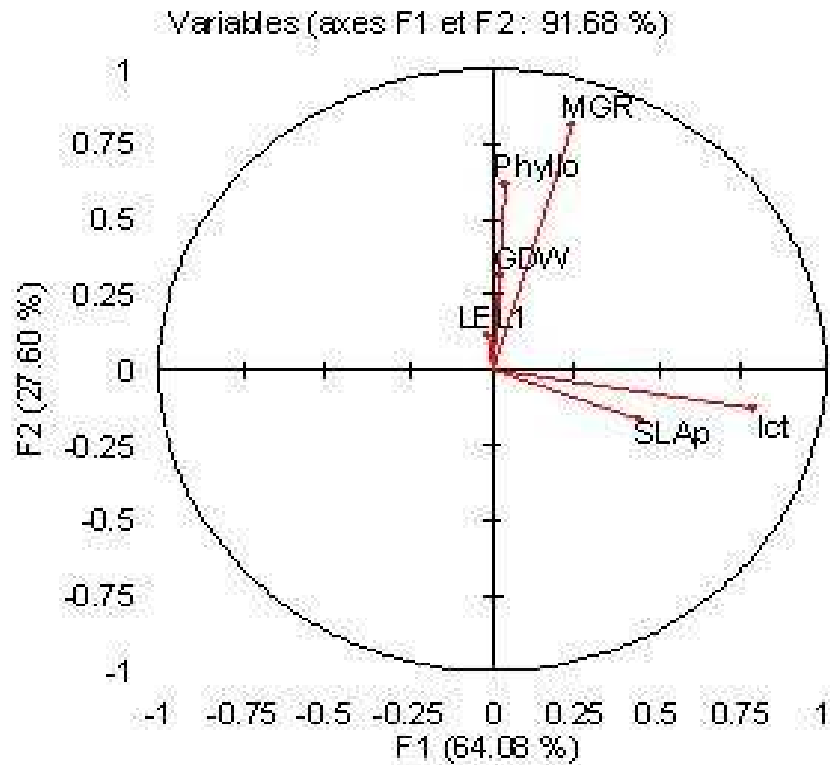
2- Starch negatively correlated with vigor based traits (SDW, LLL)

3- Sucrose positively correlated with vigor based traits

⇒ **Starch based “conservative” vs. sucrose based “bold” behavior?**

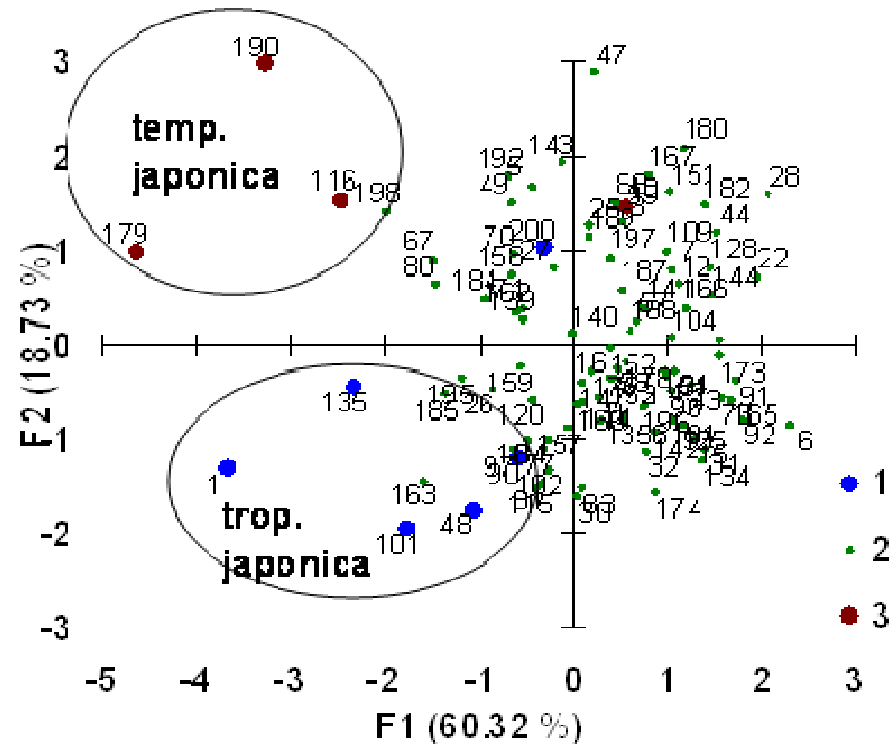
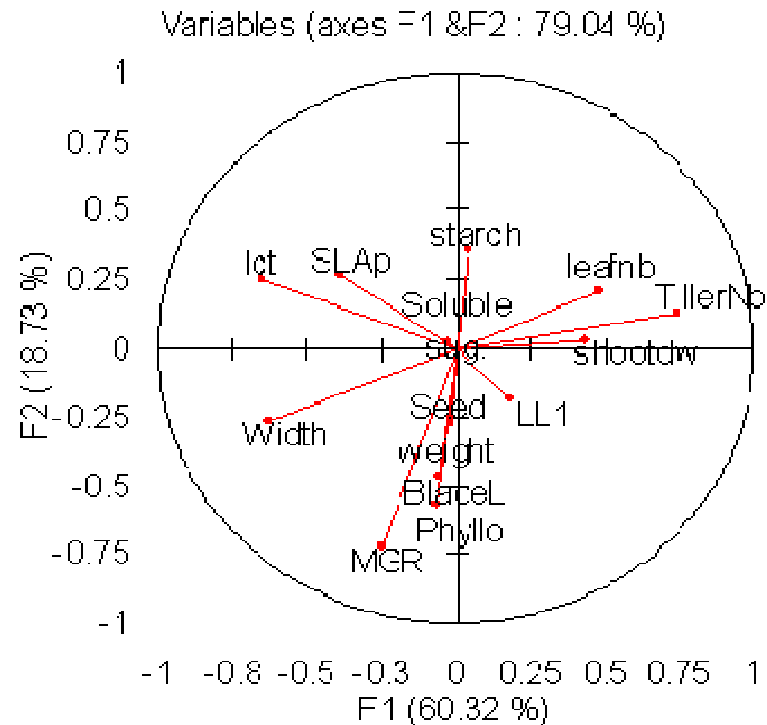
⇒ Correlation confirmed (but no significantly) between sugars and model (vigor) parameters

Factorial discriminant analysis – model parameters vs. Genetic groups



- 2 axes ~ 92% of groups explained
- Organ size & appearance (*Phyllo*, *MGR*) => similar effect as initial seed & leaf size (*GDW*, *LEL1*) (~early vigor)
- Tillering threshold (*lct*) and SLA dynamics (*SLAp*) => also similar effects
- Trop. & temp. japonica => distinct clusters

Factorial discriminant analysis – all traits vs. Genetic groups



- Vigor parameter (MGR, phyllo) negatively correlated to starch ('reasonable' behavior?)
- Tillering axis (lct) negatively correlated to leaf width (internal competition concept)
- Blade width would be more genetically explained than length
- Similar distinction of genetic groups...

Discussion & perspectives

A new step in proof of concept of model parameter

- (1) Physiological bases (sugar based plant internal competition)
- (2) Genetic bases (genetic group distinction)

Without drought treatment, difficult to evaluate parameter added value

Whole plant, model assisted phenotyping is possible

- Access to process based traits (plasticity of morphogenesis)
- Parameters as proxy for metabolic traits?
- But still exploratory

Important methodological issue: parameter optimization

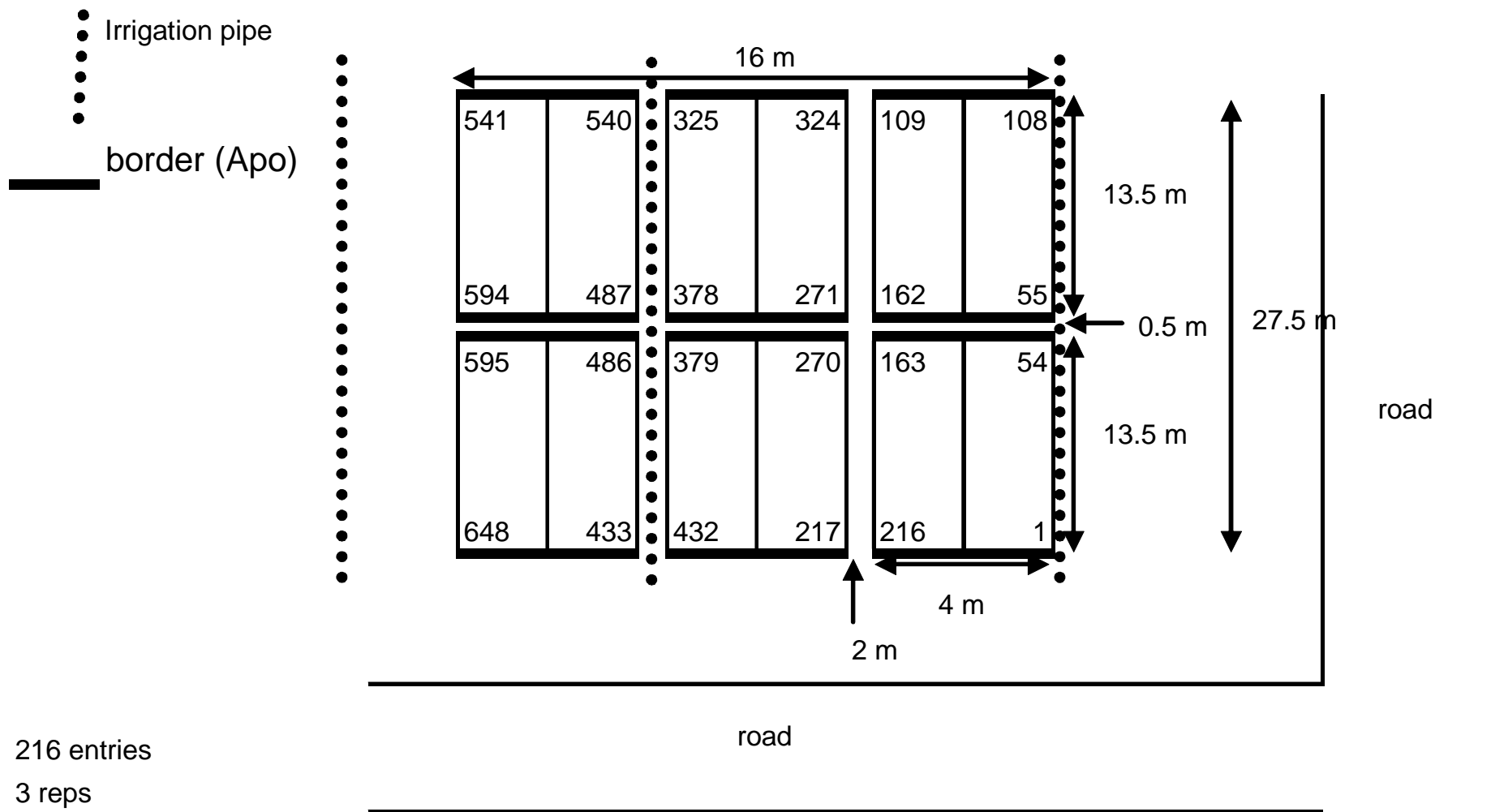
- Ecomeristem recently integrated in new software (with optimization tools)
- Which parameter to be optimized? Fixed?
- Tricky

Next steps (drought)

- Model application to populations under drought (2008-2010 project)
- Genetic association studies (cf. day3 discussion)
- Modeling of reproductive phase



Experiment Layout



Reference genotypes: Apo, IR64, Vandana

Correlation between traits model parameters vs. measurements

	LLL	LLLw	TN	SDW	Haun index	MGR	Ict	SLAp	Phyllo
LLL	1	0.24	-0.04	0.42	0.07	0.64	-0.14	-0.29	0.14
LLLw	0.24	1	-0.30	0.21	-0.07	0.48	0.15	0.06	-0.02
TN	-0.04	-0.30	1	0.63	0.31	-0.23	-0.80	-0.35	-0.40
SDW	0.42	0.21	0.63	1	0.34	0.14	-0.64	-0.33	-0.47
Haun index	0.07	-0.07	0.31	0.34	1	-0.06	-0.16	-0.20	-0.22
MGR	0.64	0.48	-0.23	0.14	-0.06	1	-0.07	-0.32	0.42
Ict	-0.14	0.15	-0.80	-0.64	-0.16	-0.07	1	0.30	0.27
SLAp	-0.29	0.06	-0.35	-0.33	-0.20	-0.32	0.30	1	-0.11
Phyllo	0.14	-0.02	-0.40	-0.47	-0.22	0.42	0.27	-0.11	1

Parameters significantly correlated
with related measured variables