

ReTraQue

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ReTraQue Vision

The vision is **to improve quality and yield faster** and more precise than conventional breeding to increase global seed market shares of the companies.

New plant Breeding Technologies (NBT) will be the key tool of future breeding to innovate new robust crop varieties, the first step in the food and feed chain. ReTraQue's vision is to **develop plant varieties more precisely and faster.**

ReTraQue has following **equally important outputs:**

- **New Plant Breeding Technologies - establish CRISPR/Cas technologies**



- **Improved prototypes with higher yield and quality**

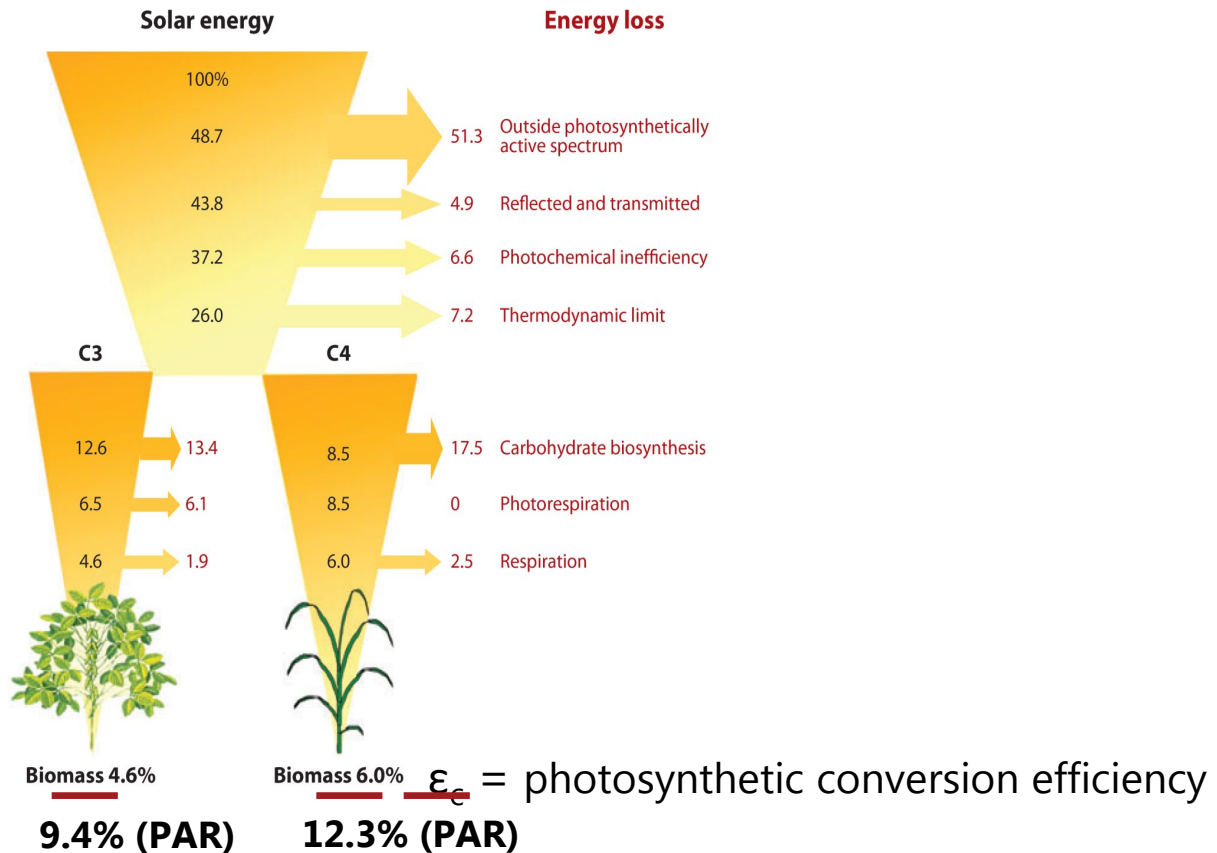
- **Photosynthesis efficiency**
- **Resistance to Fusarium**
- **Improved feed quality**



Knowledge of traits - Identify relevant gene sequences for mutation

- **Photosynthesis – improve efficiency**
 - Decrease of photosynthetic antennae
 - Adjustment of e-transport components
 - Improved CO₂ fixation, photorespiration or C-metabolism
 - Accelerate recovery from photoprotection
- **Fusarium resistance**
 - Nepenthesin – inhibit the Fusarium phytases activity, the growth is then suppressed.
 - **Prototypes developed – barley and wheat**
- **Improved digestibility**
 - Knock out specific protease inhibitors
 - Screen for natural variation in genes/enzymes for lignin synthesis
 - **Prototypes developed – barley, wheat, ryegrass and alfalfa**

Photosynthesis is surprisingly inefficient



Acknowledgement to contributors at UCPH



Improving photosynthetic conversion efficiency (ϵ_c) is the key

Yield equation:

$$Y_p = Q * \epsilon_i * \epsilon_c * \epsilon_p$$

Q= solar radiation/ unit area of land/ season

ϵ_p = harvest index

ϵ_i = light interception efficiency

ϵ_c = photosynthetic conversion efficiency

The Green Revolution increased ϵ_i and ϵ_p

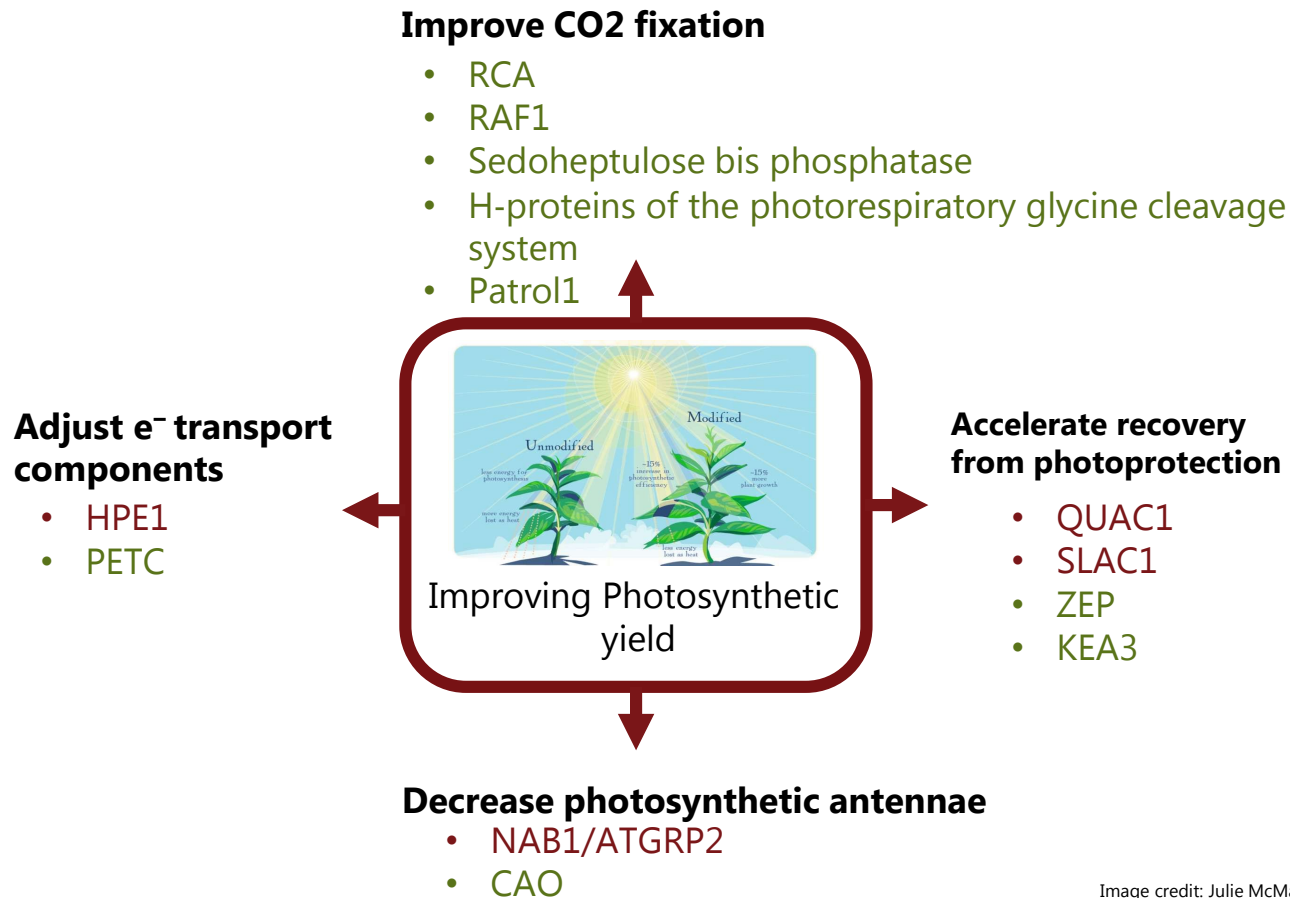
Current estimates: $Y_p = Q * 0.9 * \underline{0.02} * 0.6$

theoretical conversion efficiencies: C3 plants **0.10**

C4 plants **0.13**

Estimate: 'At least a 50% improvement in photosynthetic conversion efficiency will be critical to meet the doubled global productivity of grain crops that will likely be needed over this century'. (Zhu et al. 2010)

Photosynthetic genes affecting yield

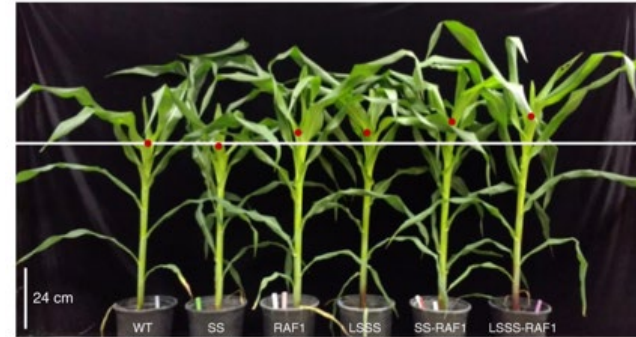


Trend Card

RAF1

Description

Encodes an ancillary chaperone protein that functions in Rubisco biogenesis. RAF1 dimers function in the assembly of the large subunit of Rubisco. Co-expression of RAF1 and *rbcl* in tobacco cells results in increased photosynthesis and plant growth.



RETRAQUE APPROACHES-

- Overexpression of Raf1 increases Rubisco content and activity , increasing plant fresh weight (growth)
- Targeted promoter mutations by CRISPR-CAS9 constructs to either increase transcription / gain of function by improving transcription factor affinity to promoter binding sites

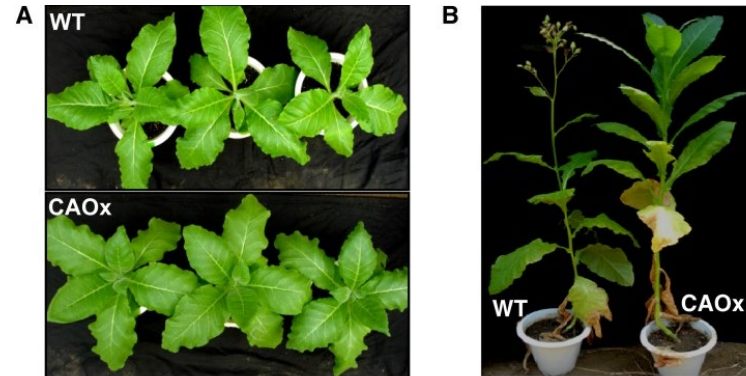


CAO

Description

Encodes chlorophyllide *a* oxygenase which converts chlorophyllide *a* to chlorophyllide *b* by catalyzing two successive hydroxylations at the 7-methyl group of chlorophyllide *a*. Mutants are deficient in pigments that associate with thylakoid membrane proteins, lacking chlorophyll *b* and light-harvesting proteins of photosystem II.

Trend Card



RETRAQUE APPROACHES-

- Overexpression of CaO modifies the size of the photosynthetic antenna, altering the wavelengths of light accessible to the plant
- Targeted genetic mutations by CRISPR-CAS9 constructs- loss of function to alter antenna size

Tanaka et al, 2001

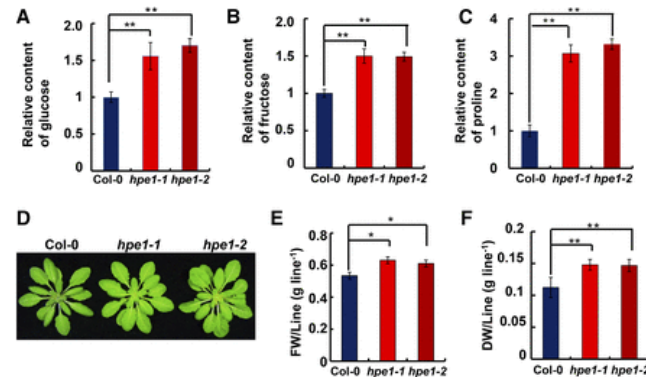


HPE1

Description

Encodes a RNA-Binding Protein RBD1.
Promotes chilling tolerance through 23S
rRNA processing.

Trend Card



RETRAQUE APPROACHES-

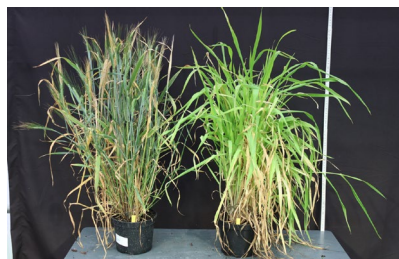
- Targeted mutations by CRISPR-CAS9 / loss of function

Deficiency of HPE1 alters the
expression of nucleus-encoded
chlorophyll-related genes



Phenotype analysis

	CaO	Raf1	HPE1
Visible phenotype	Some plants appear pale/striped. No/ delayed flowering Leaves numerous, broader than WT Plants shorter in stature	Some plants have height differences, as well as increased root mass	Plants higher than WT, Leaves darker
	<ul style="list-style-type: none"> • Leaf width • Leaf colour • Plant height • Dry weight • Tiller number • Flowering time/stage • Seed weight/ morphology 	<ul style="list-style-type: none"> • Plant height • Dry weight • Tiller number • Flowering time/stage • Seed weight/ morphology • Root biomass 	<ul style="list-style-type: none"> • Leaf width • Leaf colour • Plant height • Dry weight • Tiller number • Flowering time/stage • Seed weight/ morphology
Measurements Being analysed			
	Correlation to genotype	Correlation to genotype	Correlation to genotype



GP-6 PC5-1

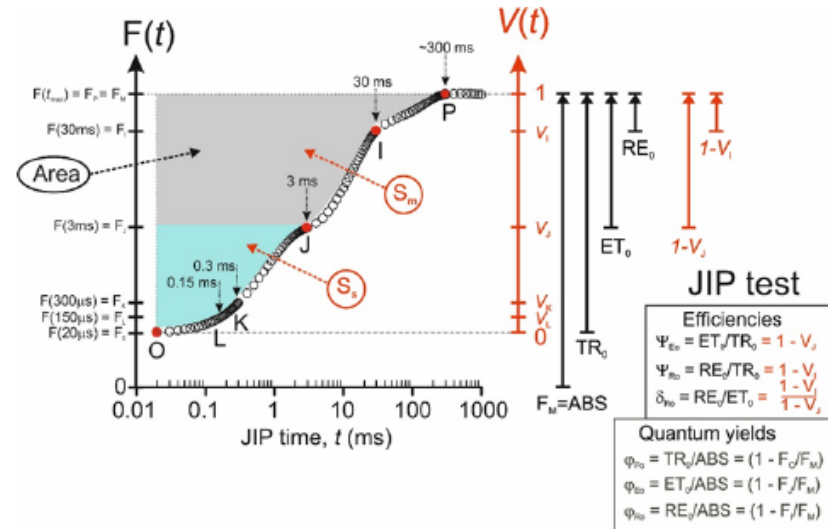


WT PR16-3



WT PH3-2 PH3-4

Pulse-amplitude modulation (PAM) fluorometry - measurements for photosynthesis efficiency

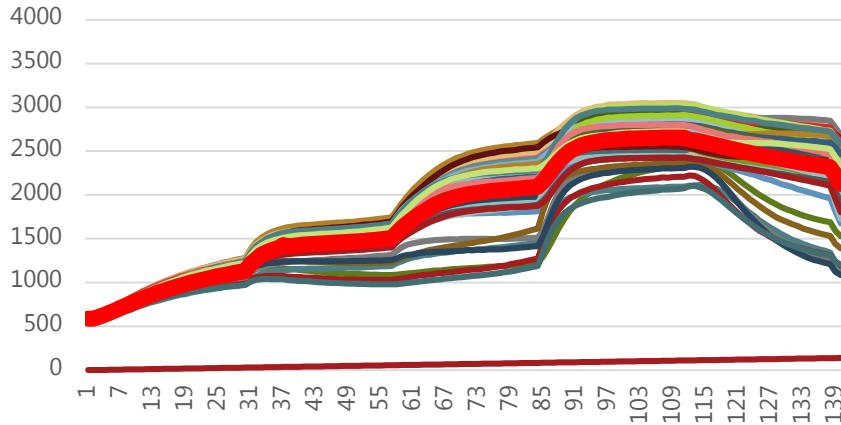


Good photosynthetic performer → high yield cultivar

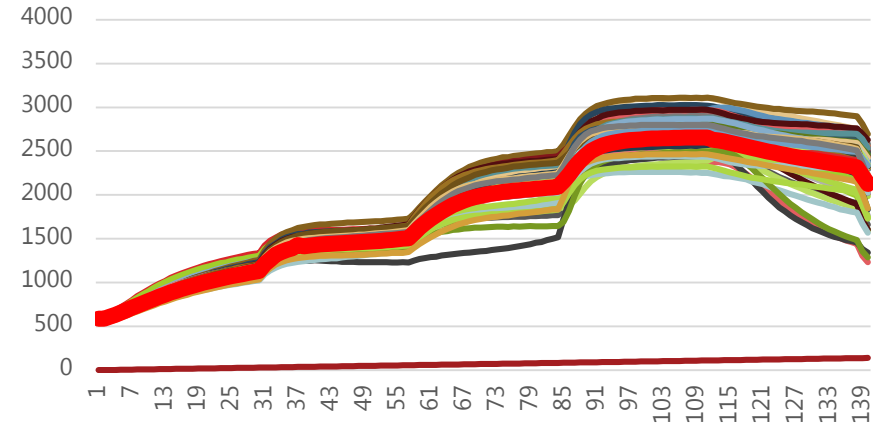
Fv/Fm: The maximum quantum yield of photosystem II (PSII) photochemistry (a key parameter indicating PSII efficiency).

PAM measurements of CRSIPR/Cas mutants - barley

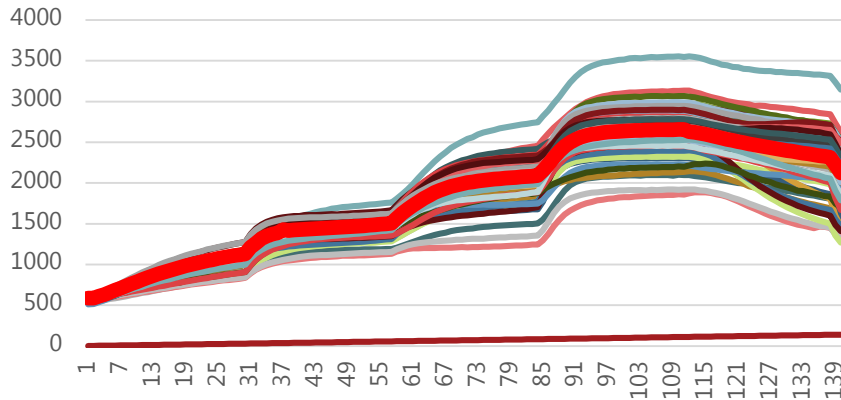
HPE – OJIP curve



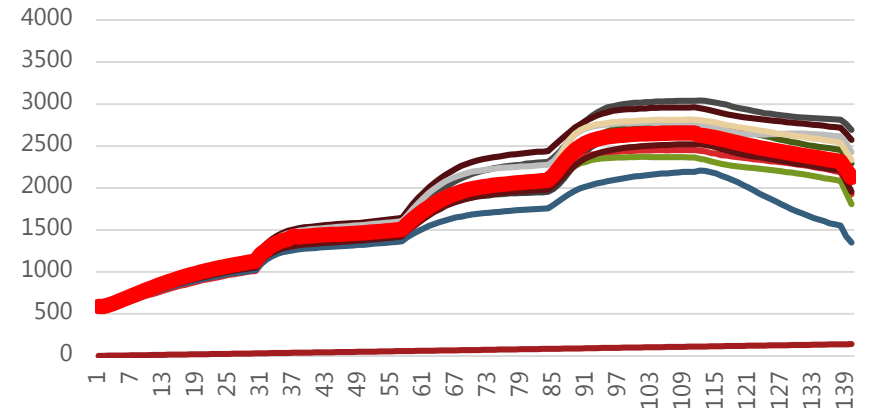
RAF – OJIP curve,



CAO – OJIP curve

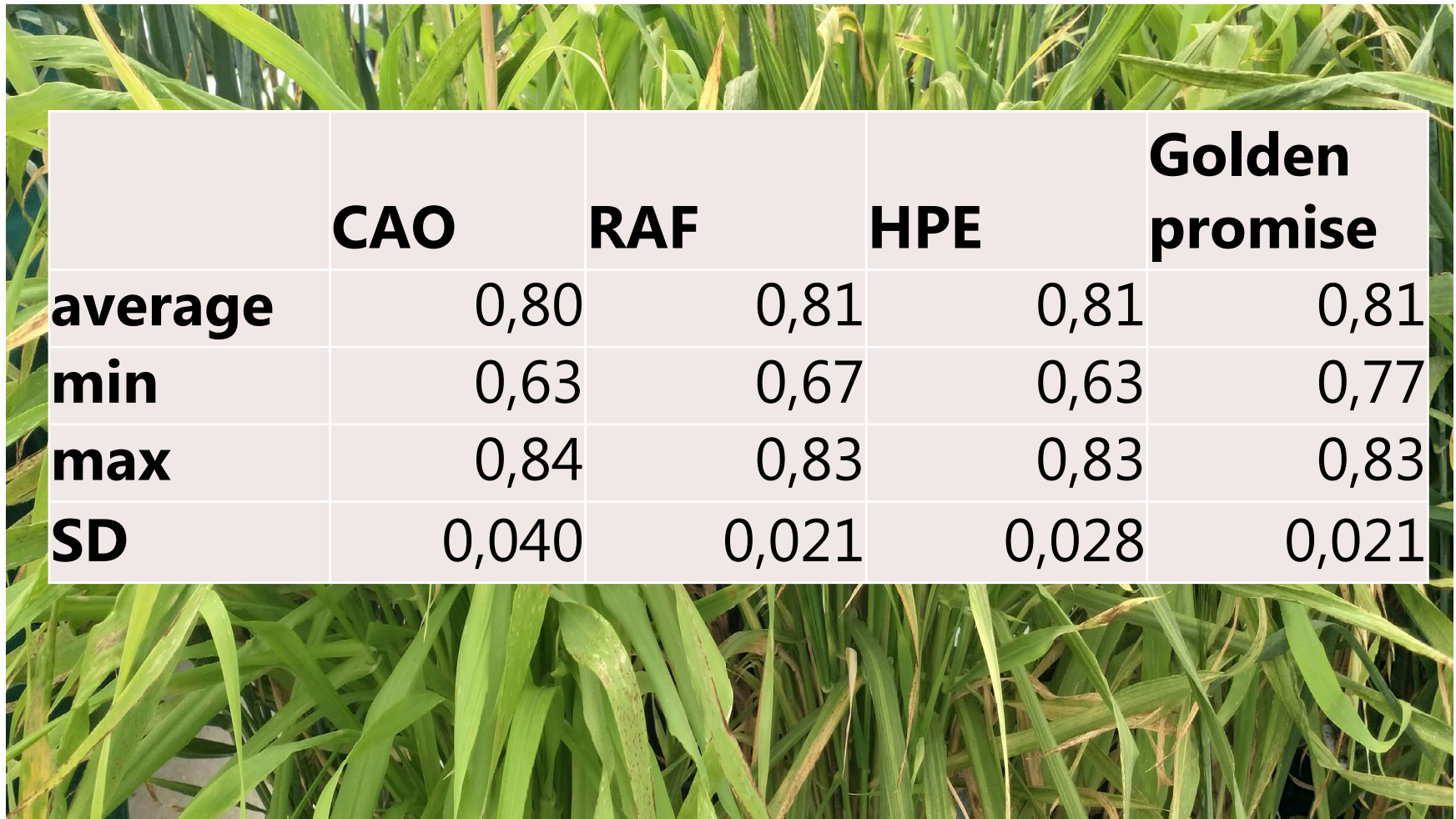


Golden promise - OJIP curve



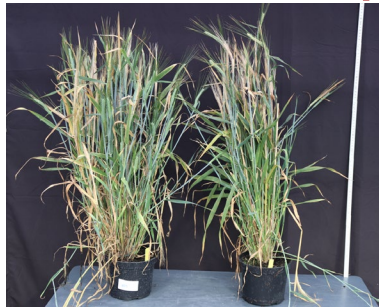
Bold red line - Golden promise average

FV/FM comparison – range

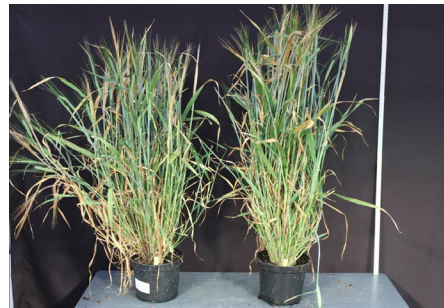


	CAO	RAF	HPE	Golden promise
average	0,80	0,81	0,81	0,81
min	0,63	0,67	0,63	0,77
max	0,84	0,83	0,83	0,83
SD	0,040	0,021	0,028	0,021

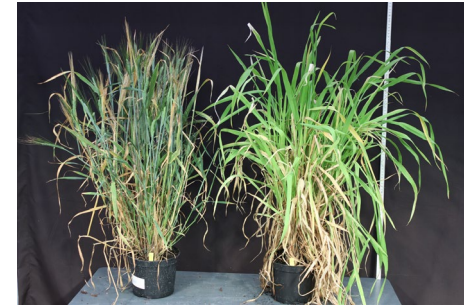
Selection of pictures- CaO mutants



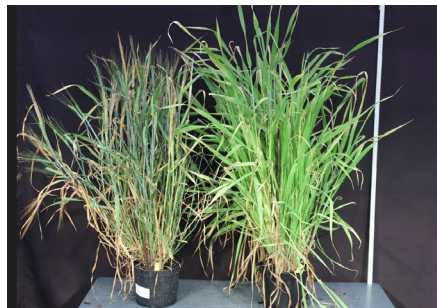
GP-6 PC 2-1



GP-6 PC 2-4



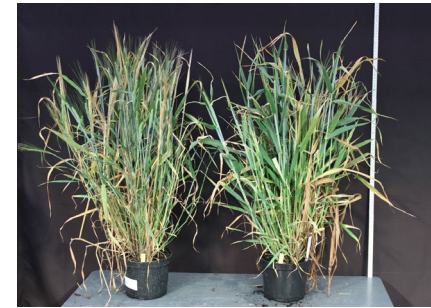
GP-6 PC 2-6



GP-6 PC4-4



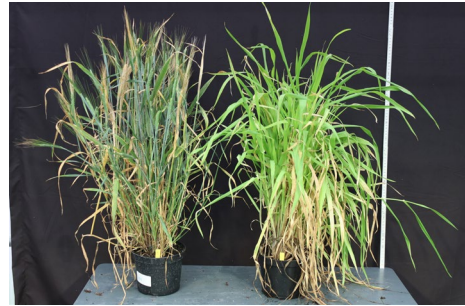
GP-6 PC 4-5



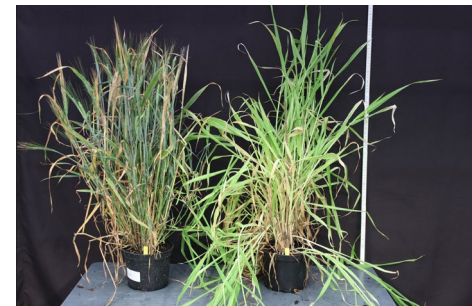
GP-6 PC4-6



GP-6 PC5-2



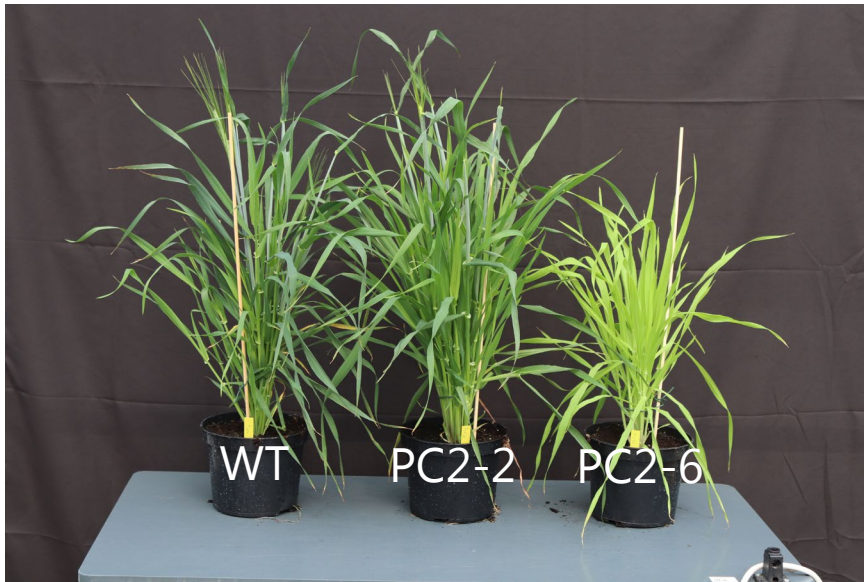
GP-6 PC5-1



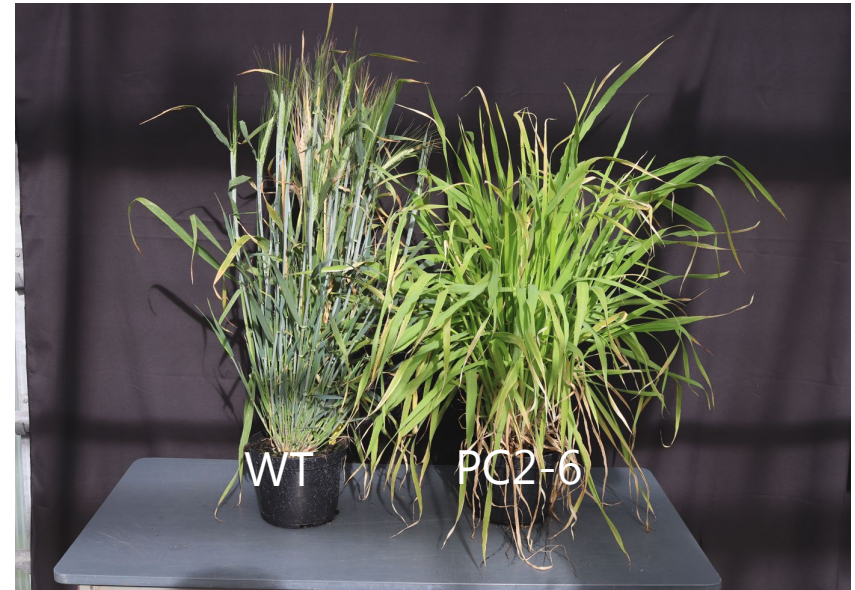
GP-6 PC5-6

CaO mutants show two distinct phenotypes – here the pale leaf phenotype

24/08/23



05/10/23

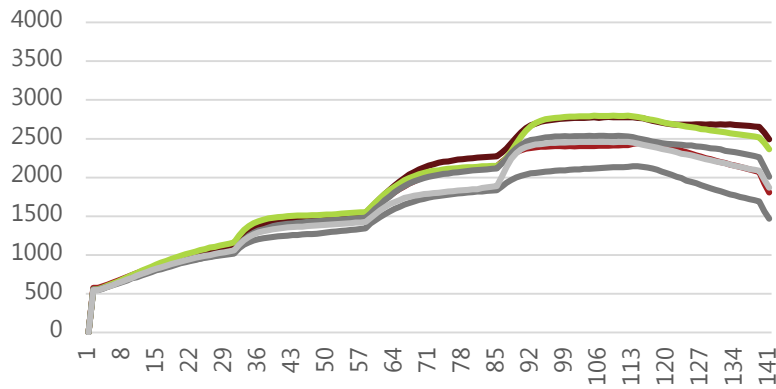


CaO – Phenotype, pale, and “Chimera” stripes

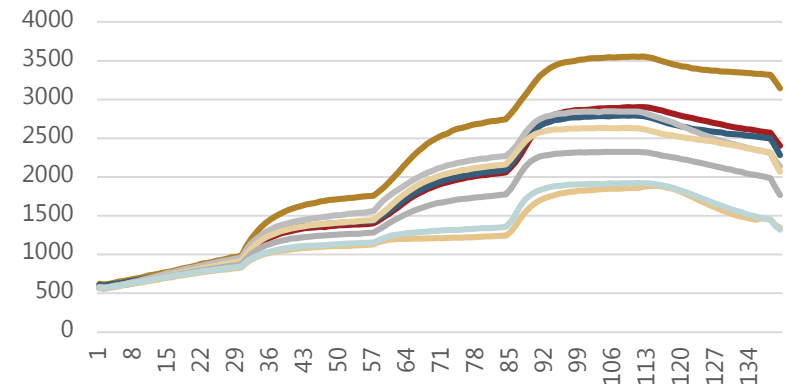


PAM measurements of CRSIPR/Cas mutants, CAO - barley

CAO chimer



CAO pale



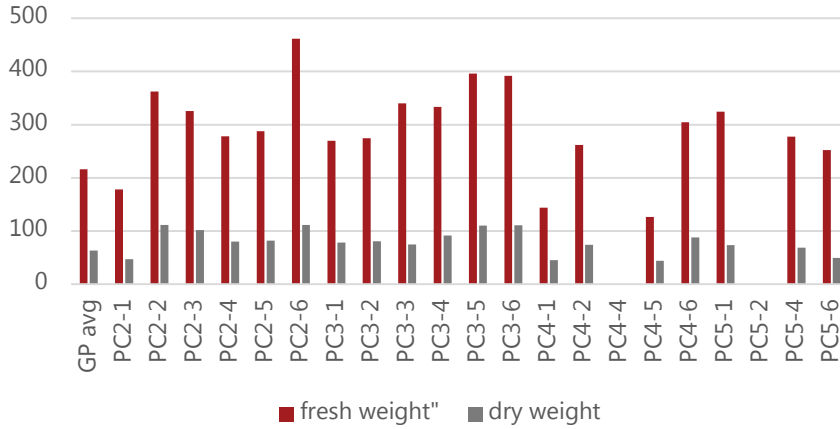
FV/FM comparison



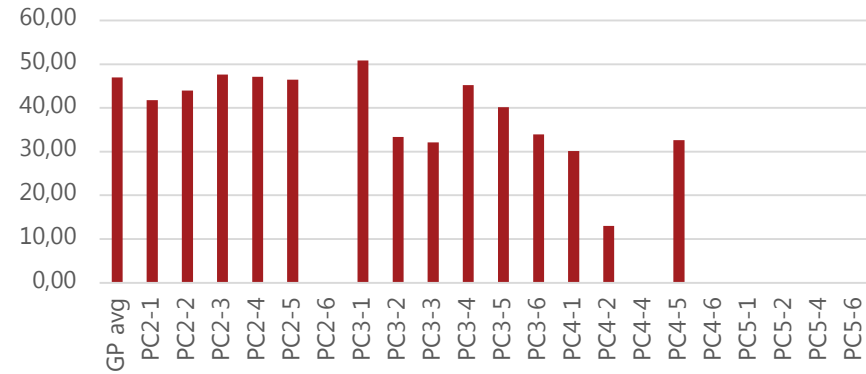
	CAO, Pale	CAO, chimer	CAO all	Golden promise
average	0,77	0,80	0,80	0,81
min	0,64	0,76	0,63	0,77
max	0,84	0,82	0,83	0,83
SD	0,055	0,017	0,036	0,021

CaO

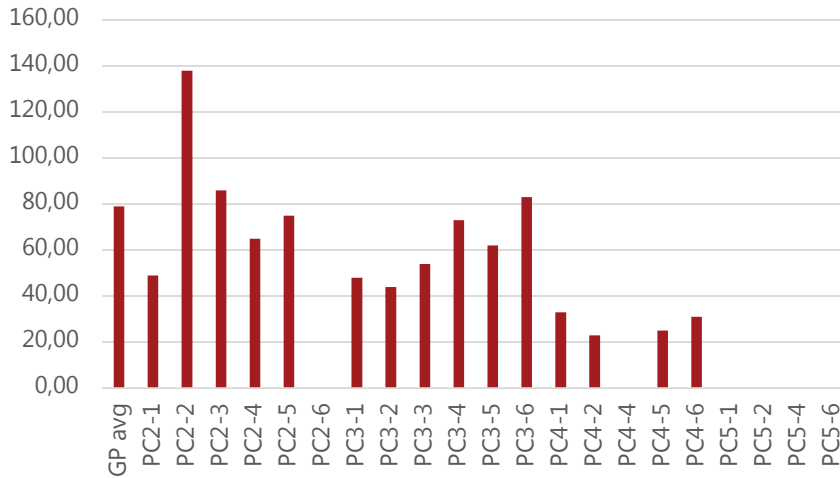
Plant Fresh weight/Dry Weight (g)



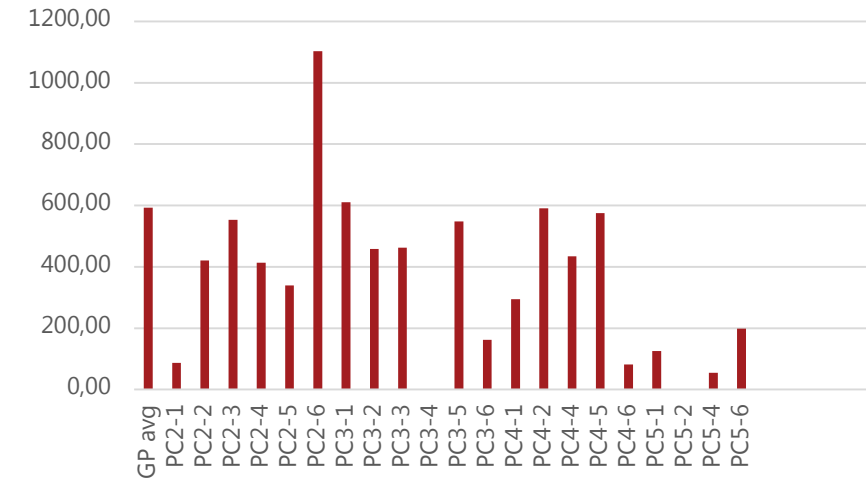
1000 seed weight (g)



number of spikes



Spike- Fresh Weight



ReTraQue has following **equally important outputs:**

- **New Plant Breeding Technologies - protocols established CRISPR/Cas**



- **Improved prototypes with higher yield and quality**
 - **Photosynthesis efficiency**
 - **Resistance to Fusarium**
 - **Improved feed quality**



Project leader status

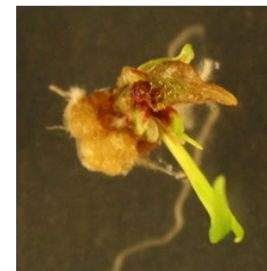
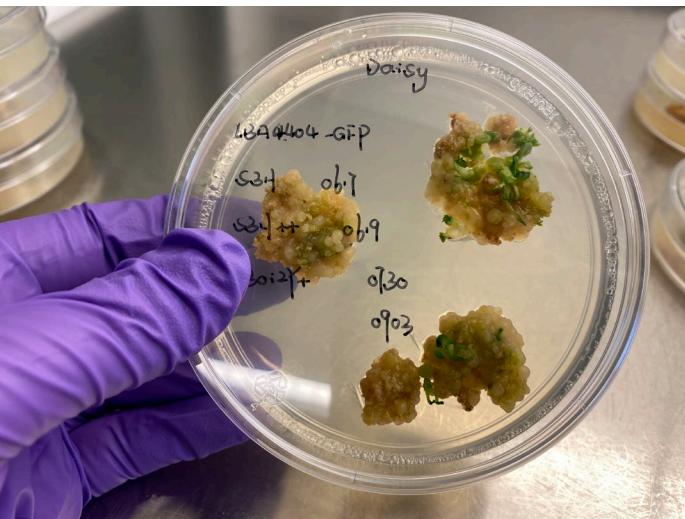
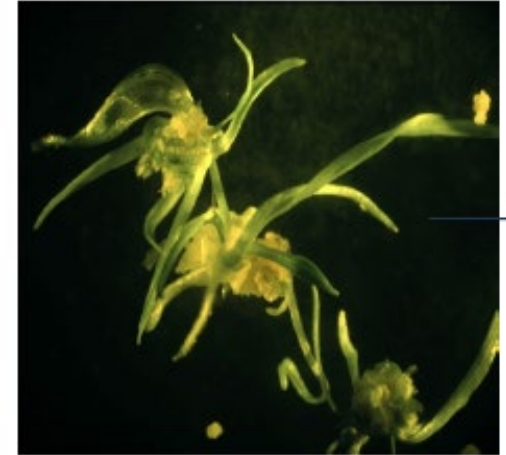
- Status overview – regeneration and transformation

Species	Regeneration and test purpose					Transformation				
	Embryo /merist	Vege- tativ	Proto plast	Gamete, ovule	Gamete, anther/ microsp.	Embryo /merist	Vege- tativ	Proto plast	Gamete, ovule	Gamete, anther/ microsp.
Alfalfa			Construct test					Stand by		
Barley					Anther/ microspore					Stand by
Potato										
Ryegrass			Construct test					Stand by		
Wheat					Anther					Stand by

Green: protocol available for several genotype, Yellow: stand by if needed to use to develop transformation protocol, Light blue: not a focus in this project.

Genotype independent transformation protocols

Barley
Alfalfa
Potato
Ryegrass
Wheat



10 wk



16 wk

CRISPR/Cas and TILLING Mutants

Species	Mutants available		
	Photosynthesis	Fusarium resistance	Digestibility
Alfalfa			Mutant/natural pop Traits has been analysed
Barley	CRISPR/Cas – Being analysed with interesting results	CRISPR/Cas - Analysed with promising results	CRISPR/Cas – Analysed with promising results
Potato			
Ryegrass			Mutant/natural pop Traits has been analysed
Wheat	Mutant pop – Being analysed	CRISPR/Cas	

Additional in house mutation populations for both barley and wheat was established

ReTraQue – protocols, prototypes and mutation population to be used in the future

✓ **New Plant Breeding Technologies - protocols established CRISPR/Cas**



- **Improved prototypes with higher yield and quality**
 - **Photosynthesis efficiency**
 - ✓ **Resistance to Fusarium**
 - ✓ **Improved feed quality**



On top of this – Mutation populations in wheat and barley

ReTraQue

Unique knowledge sharing – creating synergy

