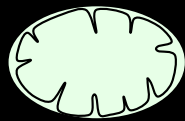


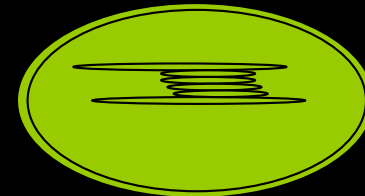
Bielefeld - 09.01.07

Mitochondria-chloroplasts crosstalks during light acclimation and stress response in tobacco leaves.



Pierrick PRIAULT

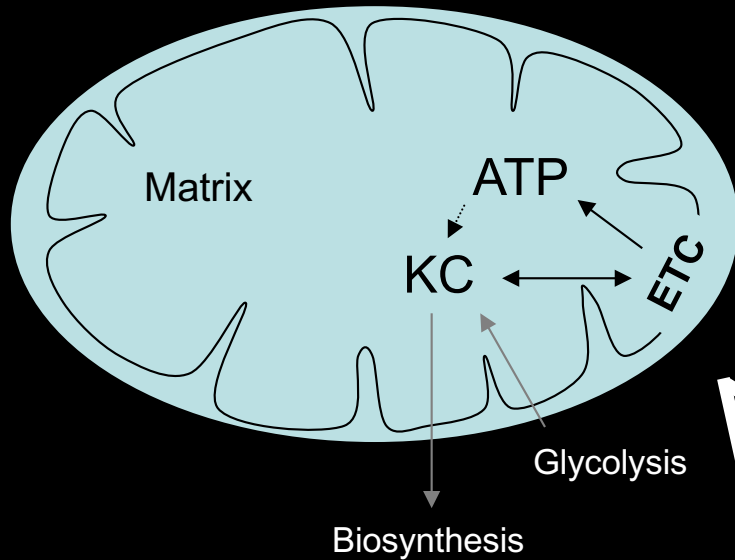
Univ. Paris Sud, France



INTRODUCTION

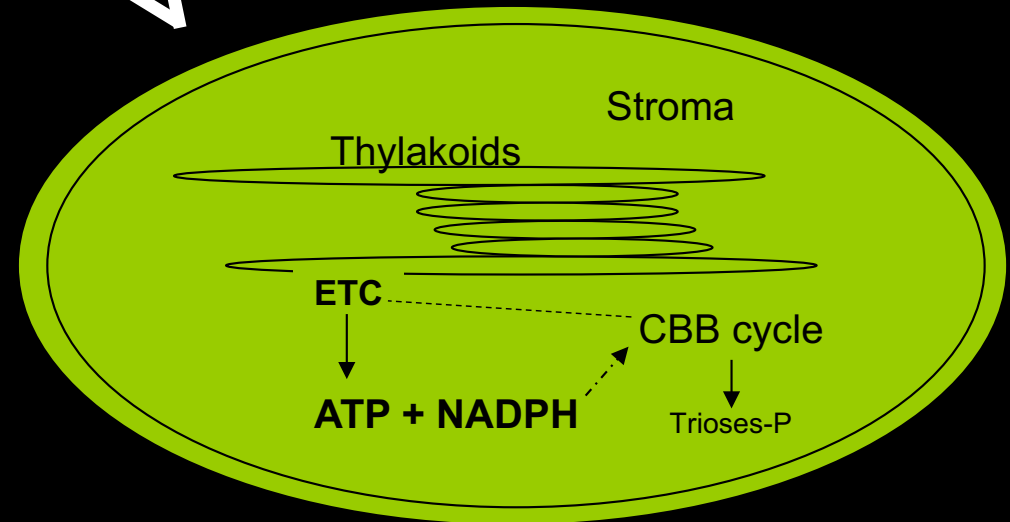
1.1 Mitochondria/chloroplasts

Mitochondria



Mito/chloro interactions basis
Metabolites, energy, reducing
power exchanges

+



ETC: Electron transport chain
KC: Krebs Cycle
CBB cycle: Calvin-Benson-Bassham cycle

Chloroplasts

Mitochondria



Use of specific inhibitors of mitochondrial metabolism:
oligomycin, antimycin A, SHAM, ... (Krömer et al., 1988;
Padmasree et Raghavendra, 2003; ...).

Chloroplastes

Mitochondria



+

Substrates supply of mitochondrial metabolism.

Chloroplasts

Mitochondria function in optimizing photosynthesis is also important under stress environments such as **high light or low temperature** (Saradevi and Raghavendra, 1992; Hurry et al., 1995).

In high light, mitochondria can oxidize the excess of reducing equivalents generated by photosynthesis, preventing overreduction of chloroplastic redox carriers and thus maintaining high rates of photosynthesis (Krömer, 1995; Padmasree and Raghavendra, 2000).

Objectives

Model plant

Nicotiana sylvestris

**Acclimation to different growth
light intensities.**

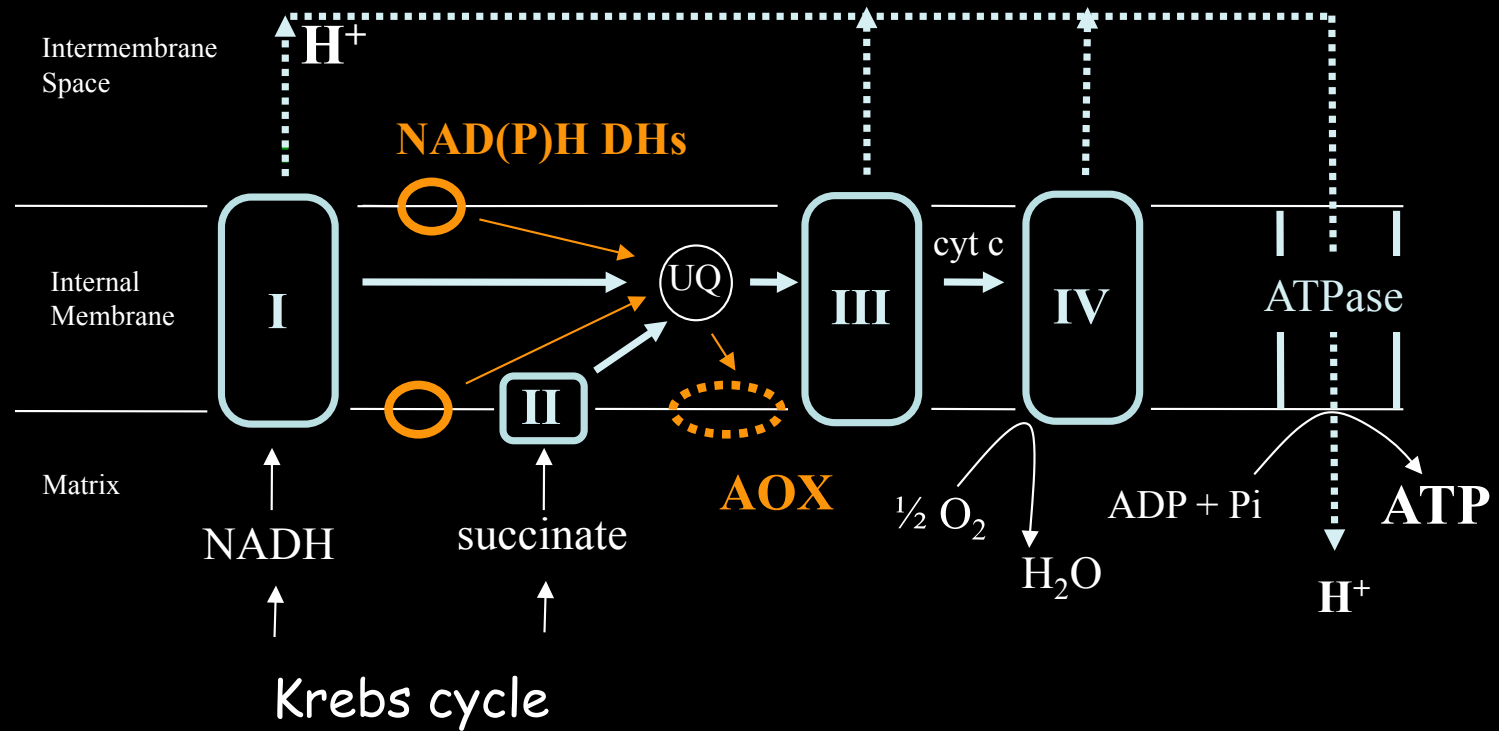
= light acclimation

**Abiotic stress responses.
herbicide-induced**



INTRODUCTION

1.3 *Mito. electron transport chain*

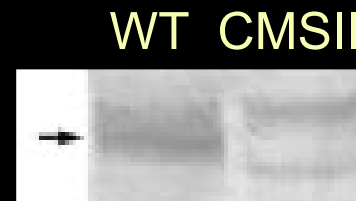
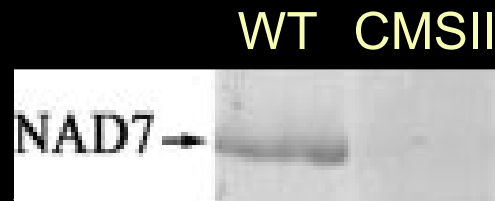


Mitochondrial electron transport chain.

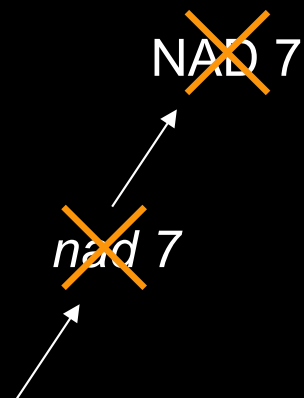
1.4 *N. sylvestris* CMSII mutant

Isolated mitochondria

Leaf discs



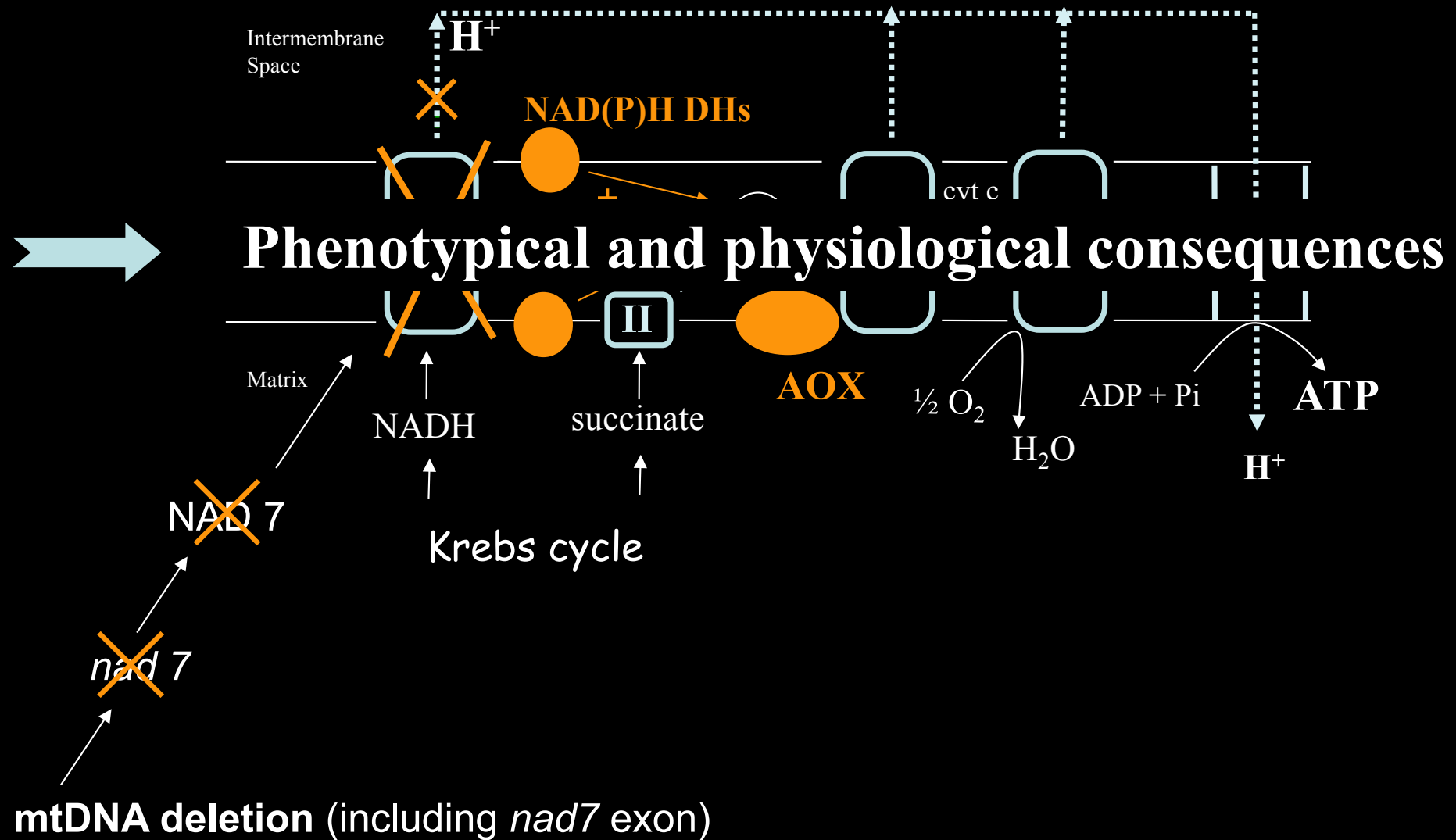
Pineau et al., 2005



mtDNA deletion (including *nad7* exon)

INTRODUCTION

1.4 *N. sylvestris* CMSII mutant



INTRODUCTION

1.4 *N. sylvestris* CMSII mutant

Lower growth rate and adult size.

Cytoplasmic Male Sterile plant



INTRODUCTION

1.4 *N. sylvestris* CMSII mutant



Lower growth rate and adult size.

Cytoplasmic Male Sterile plant

Higher dark respiration rate
Similar photosynthetic capacity

but

Lower net carbon assimilation in
ambient conditions

**Higher constitutive antioxidant
components and higher
resistance to biotic and abiotic
stress** (Dutilleul et al., 2003a).

Objectives

Model plant

Nicotiana sylvestris

**Acclimation to different
growth light intensities.
= light acclimation**

**Abiotic stress responses.
herbicide-induced**



Responses of metabolism and/or photosynthesis to light conditions by phenotypic plasticity, which tend to optimize the balance between light absorption and its use.

2.1 *Definitions*

LL-grown plants



HL-grown plants

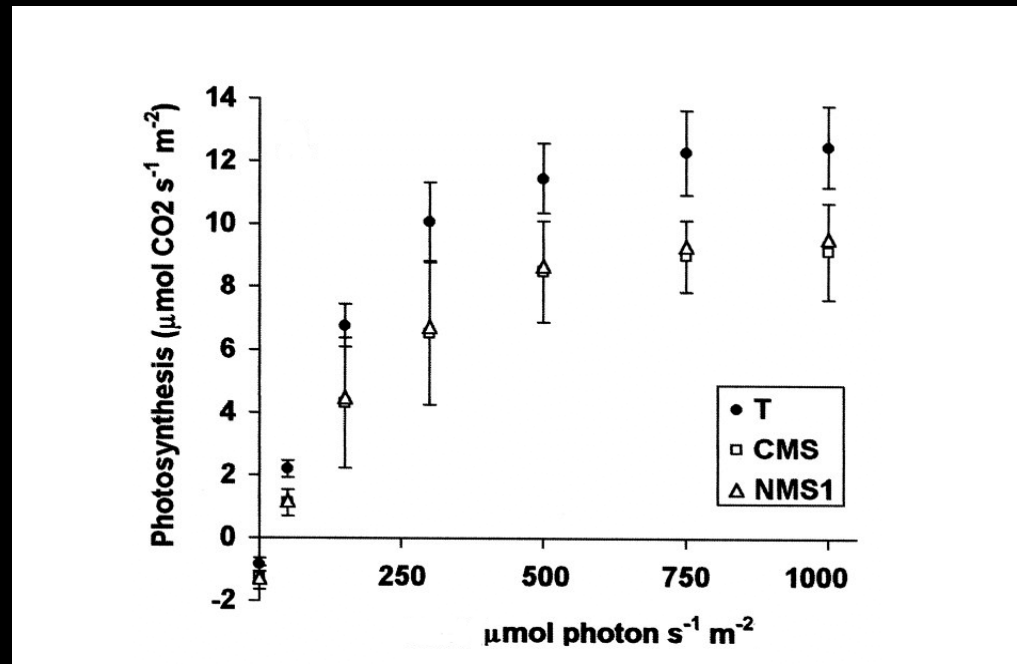


Mitochondria involvement ?

2.2 Preliminary results

Lower net carbon assimilation was observed in CMSII plants in several CO₂ and light conditions

Greenhouse-grown plants: Sabar et al., 2000; Dutilleul et al., 2003b.

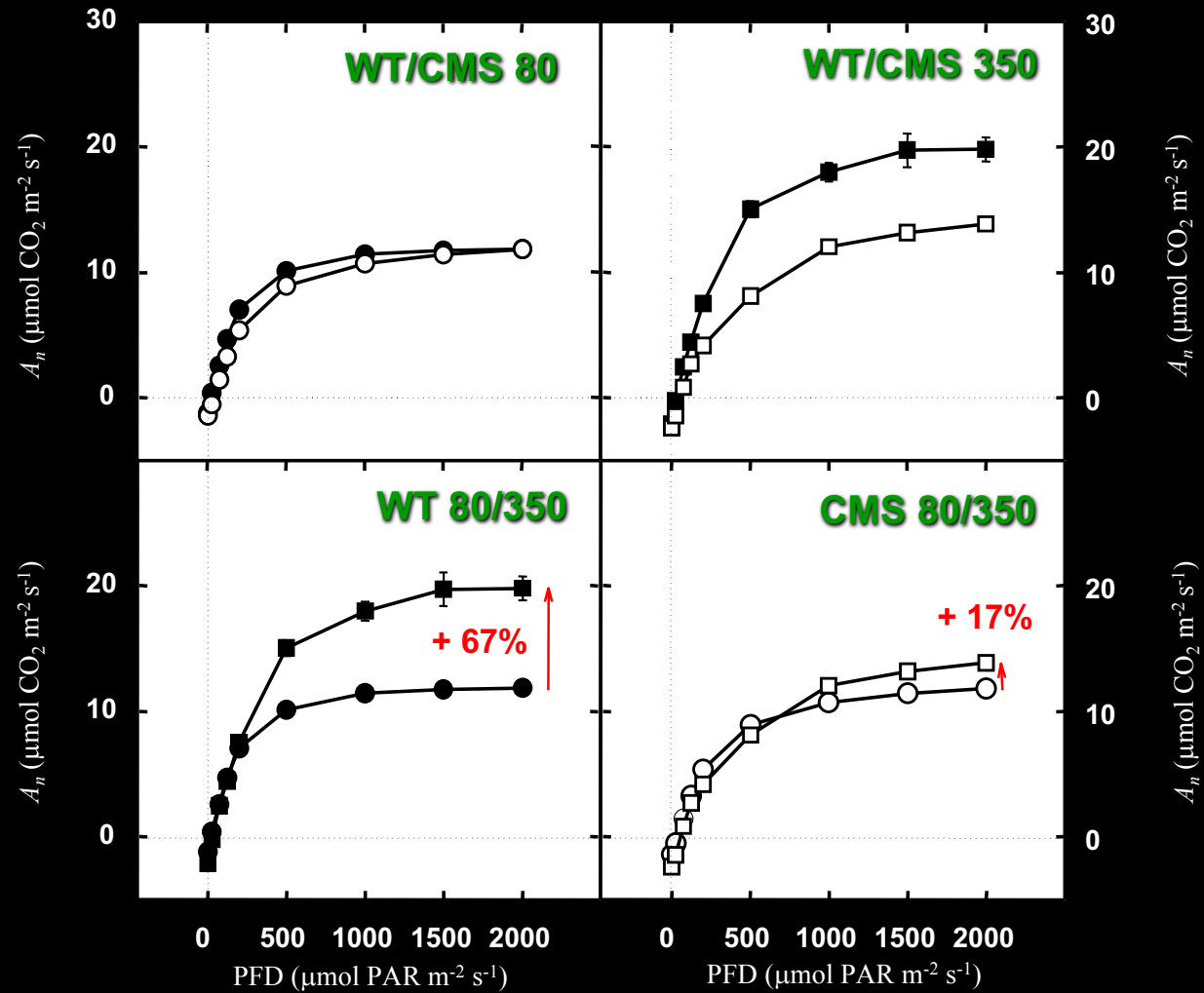
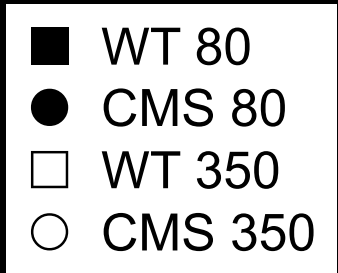


Sabar et al., 2000

↪ Plants were grown either under 80 or 350 µmol PAR m⁻² s⁻¹.

LIGHT ACCLIMATION

2.2 Results: light response curves

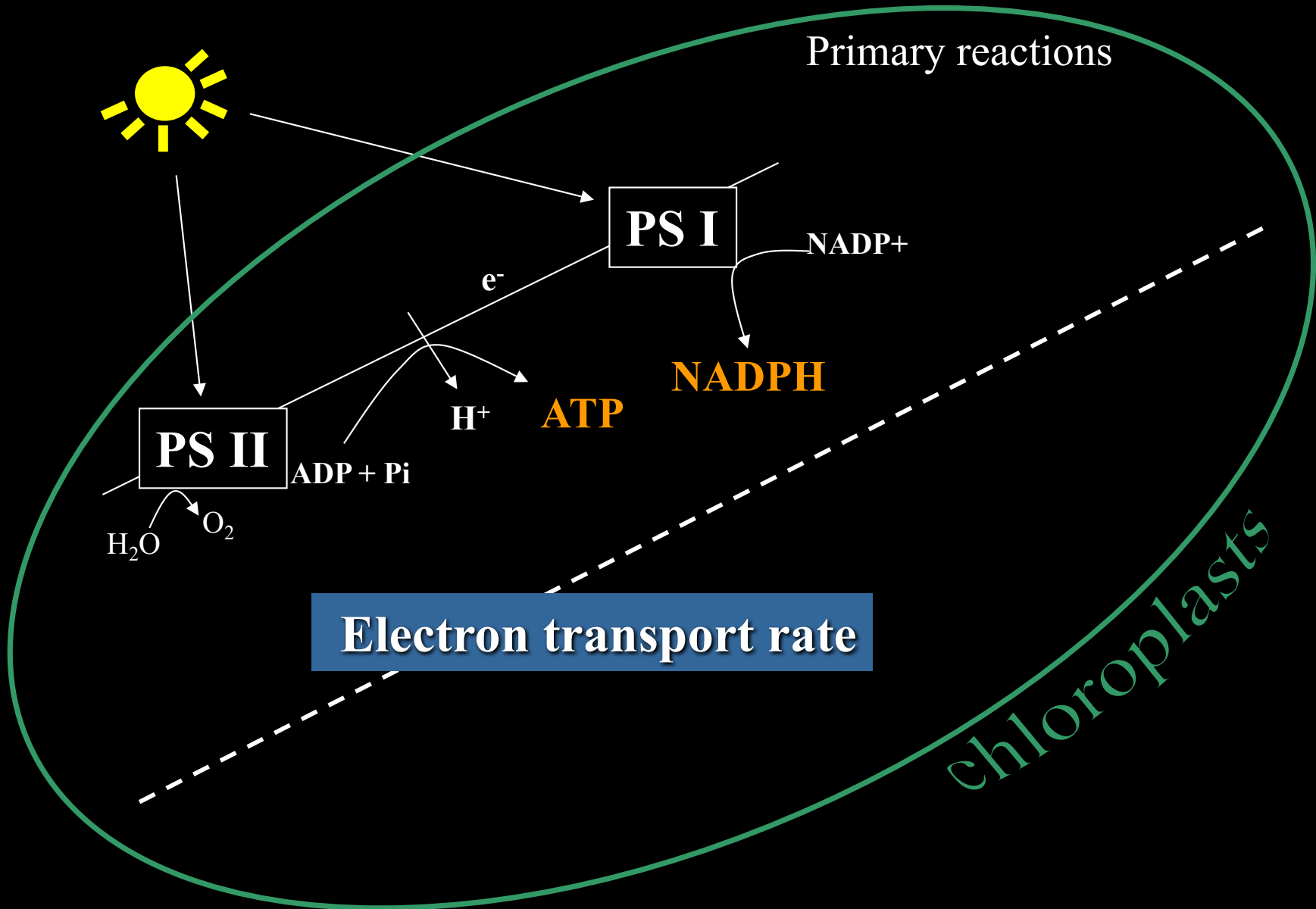


Identifying the mechanisms which impair adjustment of net carbon assimilation to an increased growth light intensity in mutant plants.

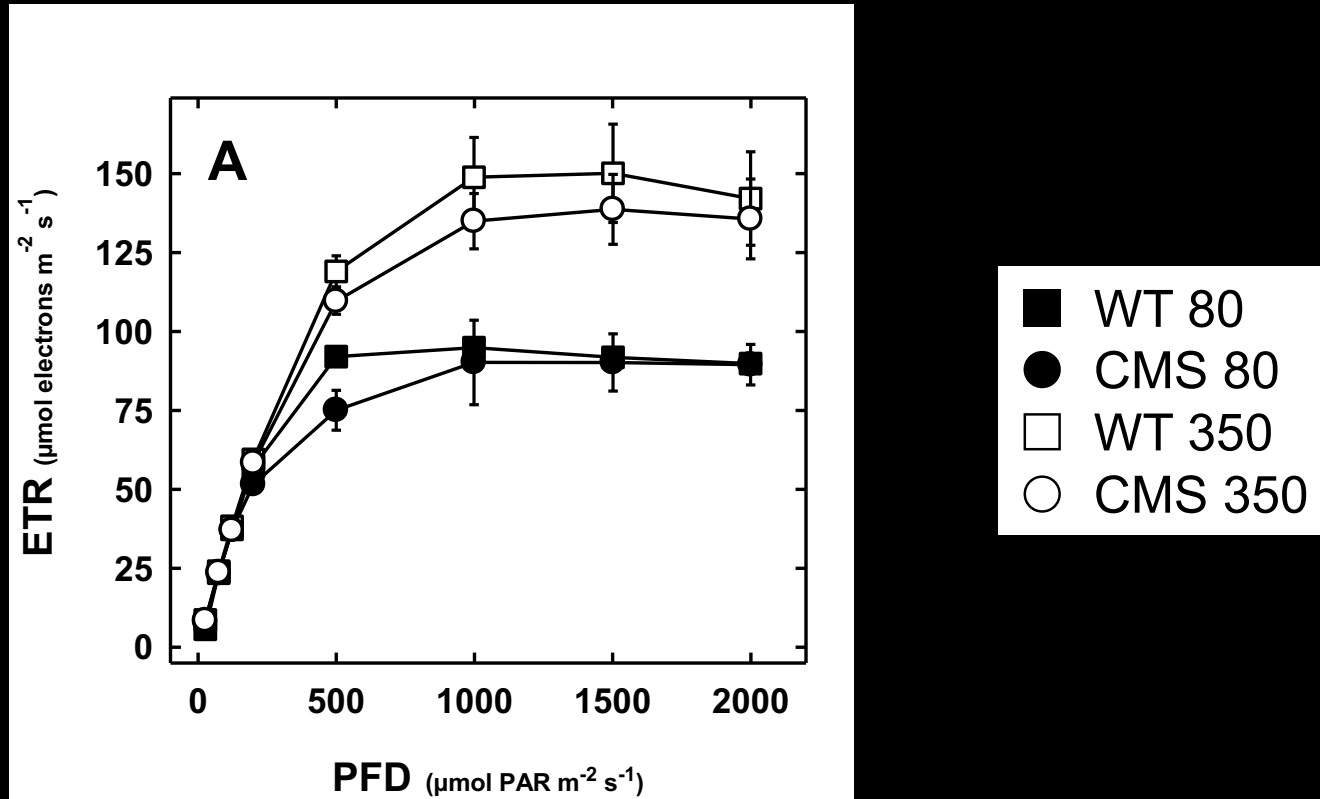


Primary reactions of photosynthesis ?

2.4 Primary reactions



Total linear electron transport rate



Primary reactions of photosynthesis are not a limiting factor for the non acclimation of the net CO₂ assimilation in HL-CMS plants.

but CMS 350 plants exhibit an excess of electrons, not used for assimilation.

in CMS 350 plants:

Lower A_n

Similar ETR

Where go excess electrons in CMS 350 ?

A_n (WT 350) - A_n (CMS 350) decreases under increased photorespiration in CMS 350? non-photorespiratory conditions (e.g. high light, high CO_2 , low O_2).

Glycolate Oxidase activity is higher in CMS 350 plants.

2.5 *Increased photorespiration ?*

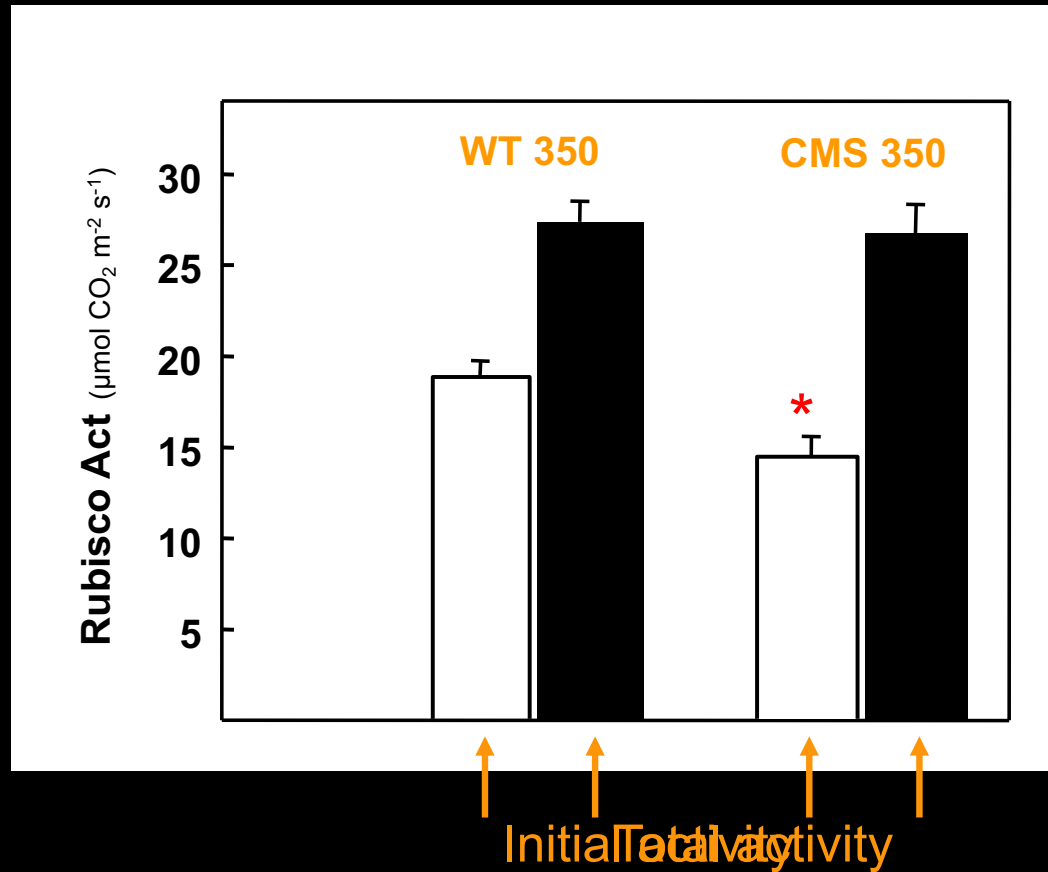
↓
Increased photorespiration in CMS 350 ?

↙ ↘
Specificity factor of Rubisco ?

Lower CO₂ concentration in chloroplasts ?

↓
Rubisco activity measurements

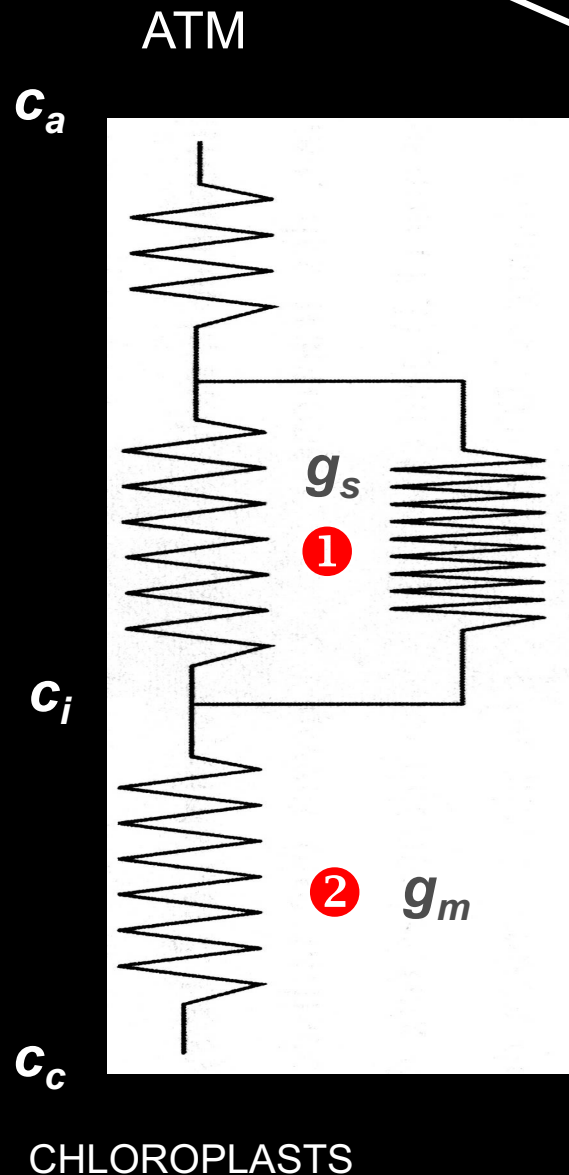
2.5 Rubisco activity



In HL-CMSII plants, Rubisco was not affected in terms of its capacity but presents a lower initial activity, which accounted for the lower net carbon assimilation.

LIGHT ACCLIMATION

2.6 CO₂ diffusion limitations



Substrate supply: CO₂ diffusion ?

2 steps were investigated:

- Stomatal limitation

(including CO₂ diffusion from the atmosphere to the substomatal cavities)

- Internal limitation

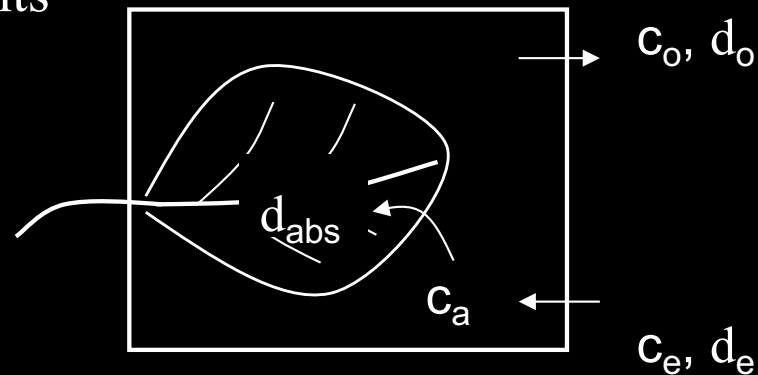
(including CO₂ diffusion from intercellular spaces, its dissolution in aqueous phase and its diffusion to the stroma inside the chloroplasts)

- Stomatal limitation

Despite a lower stomatal conductance in CMSII plants as compared to WT, for a same external CO_2 concentration, WT and CMSII plants showed similar intercellular CO_2 concentration.

- Internal limitation

Isotopic measurements



LIGHT ACCLIMATION

2.6 CO₂ diffusion limitations

- Internal limitation

$$\Delta_{(i)} = a \cdot (c_a - c_i) / c_a + b \cdot (c_i / c_a)$$

stomata

Rubisco

$$\Delta_{obs} = a \cdot (c_s - c_i) / c_a + a_i \cdot (c_i - c_c) / c_a + b \cdot c_c / c_a - d$$

stomata

Internal conductance

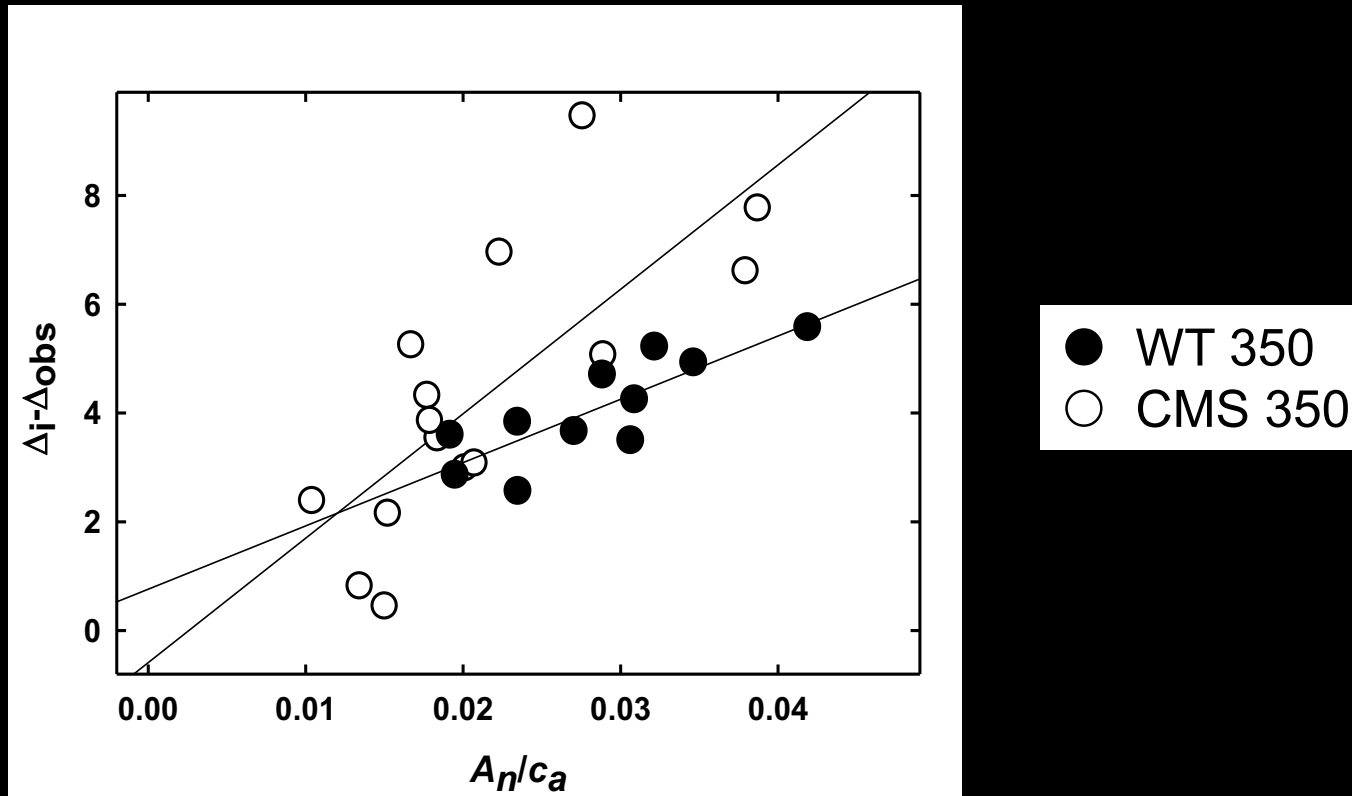
Rubisco

Photoresp
Resp

$$\Delta_i - \Delta_{obs} = (b - a_i) / g_m \cdot A_n / c_a + d$$

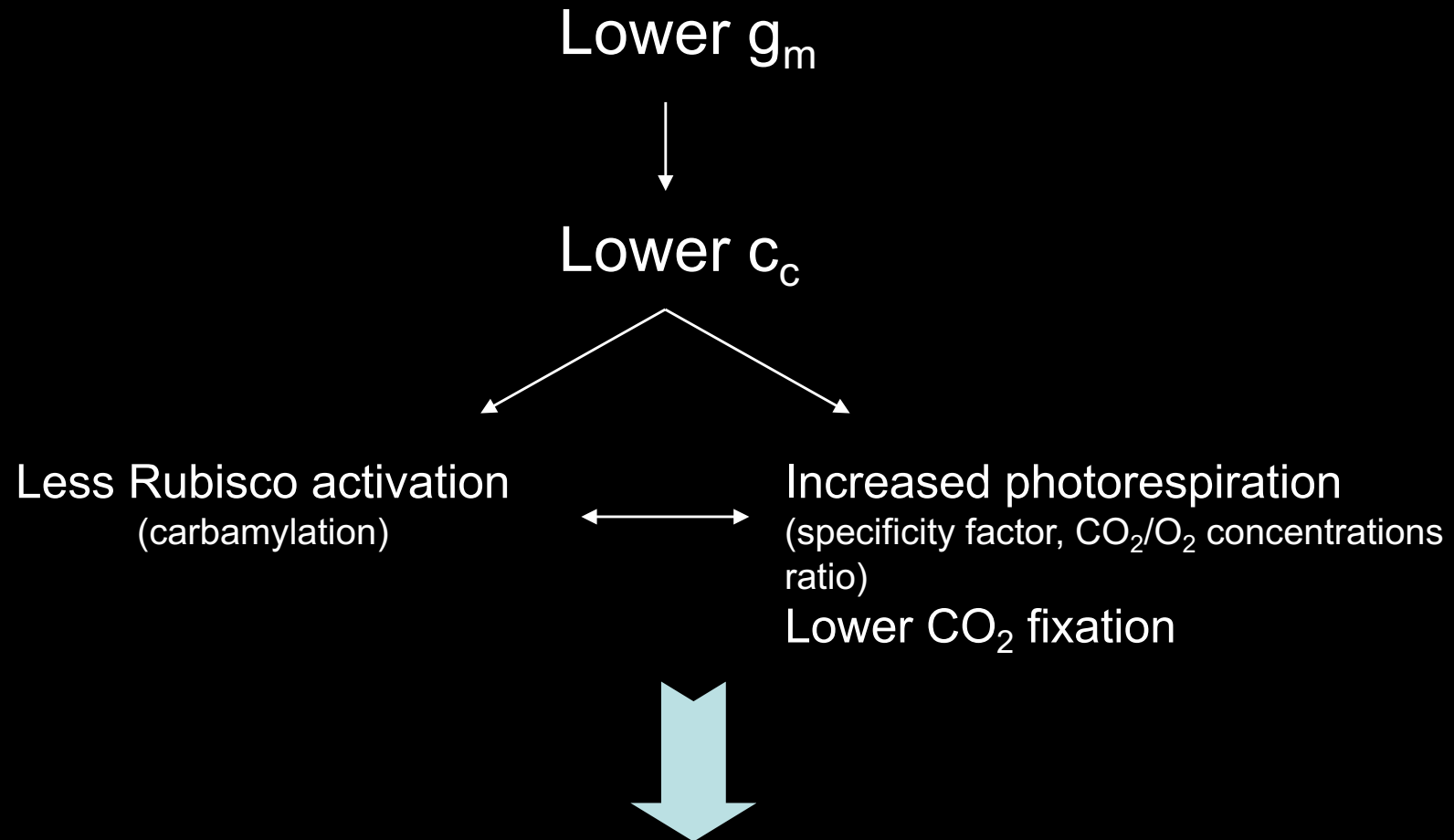
as: $A_n = g_s (c_a - c_i) = g_m (c_i - c_c)$,

- Internal limitation



Internal conductance is two times lower in CMS 350 plants than in WT 350.

2.6 *CO₂ diffusion limitations*



Two experiments were performed to confirm this result.

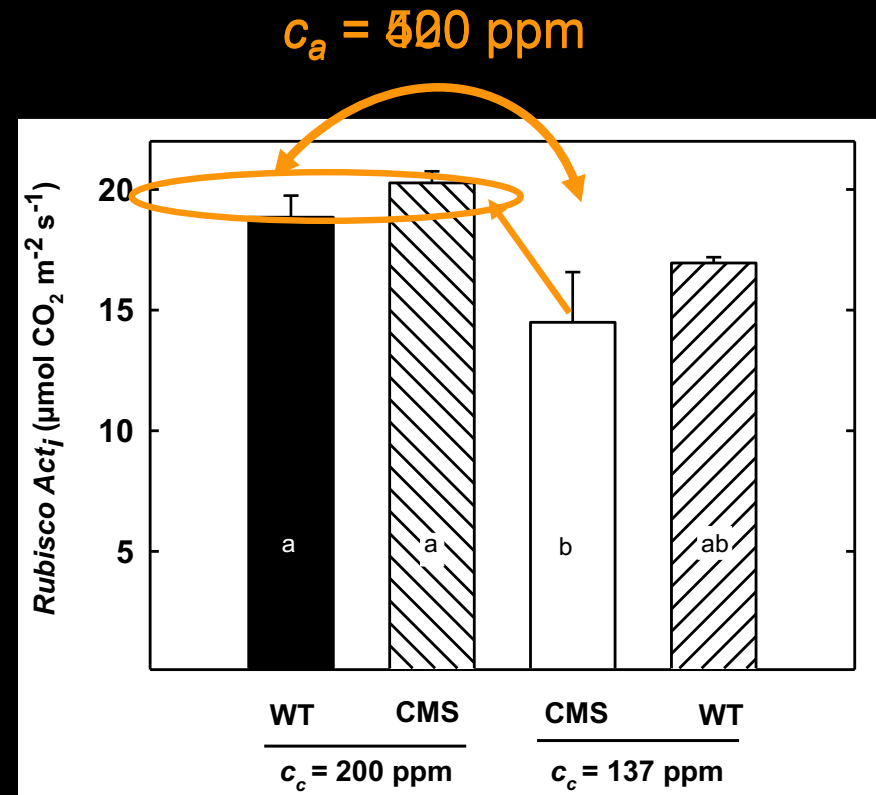
Exp. 1: growth under high CO₂



Growing CMSII plants under **high CO₂** overcome their retarded leaf growth and lower photosynthetic activity.

Exp. 2: short incubations under various [CO₂]

Aim: to adjust chloroplastic CO₂ concentration in WT and CMSII at the same level:



CO₂ supply to catalytic sites of Rubisco inside chloroplasts constitute the critical point for the non adjustment of net carbon assimilation and initial Rubisco activity in mutant plants grown under higher light intensity (*Priault et al., 2006a,b*).

Objectives

Model plant

Nicotiana sylvestris

Acclimation to different growth
light intensities.

= light acclimation

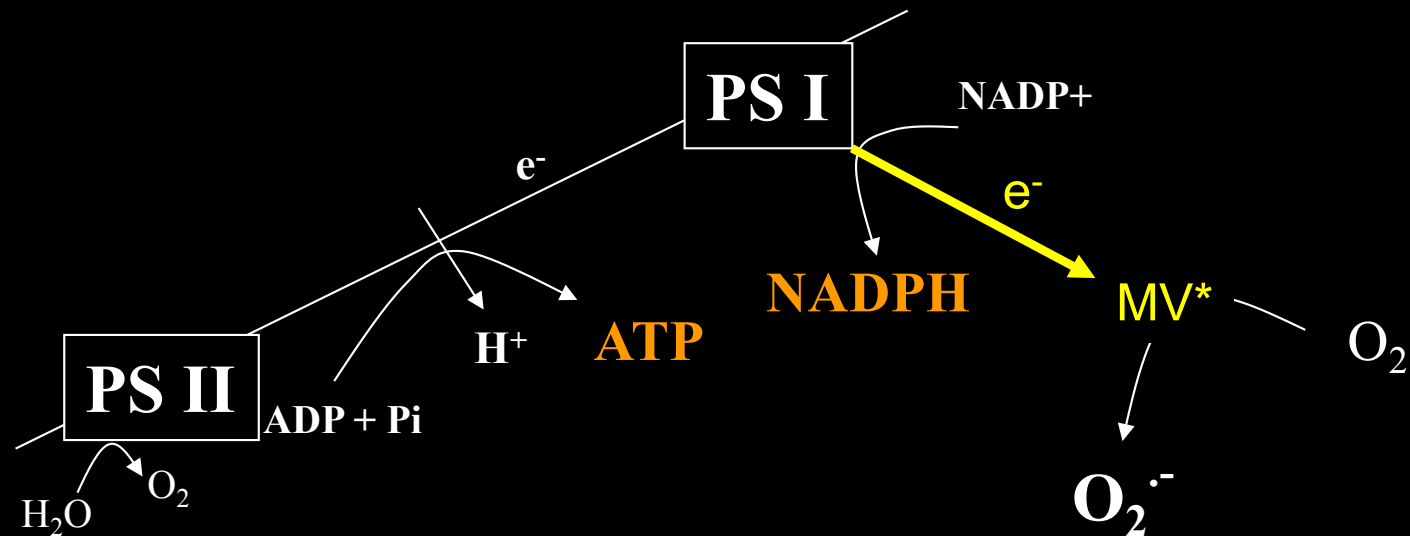
Abiotic stress responses.

herbicide-induced



3.1 Definitions

Use of an herbicide: **methylviologen**, which is commonly used in plant research to induce oxidative stress.



1. PSII integrity

2. Chlorophyll content (photobleaching)

MV damages

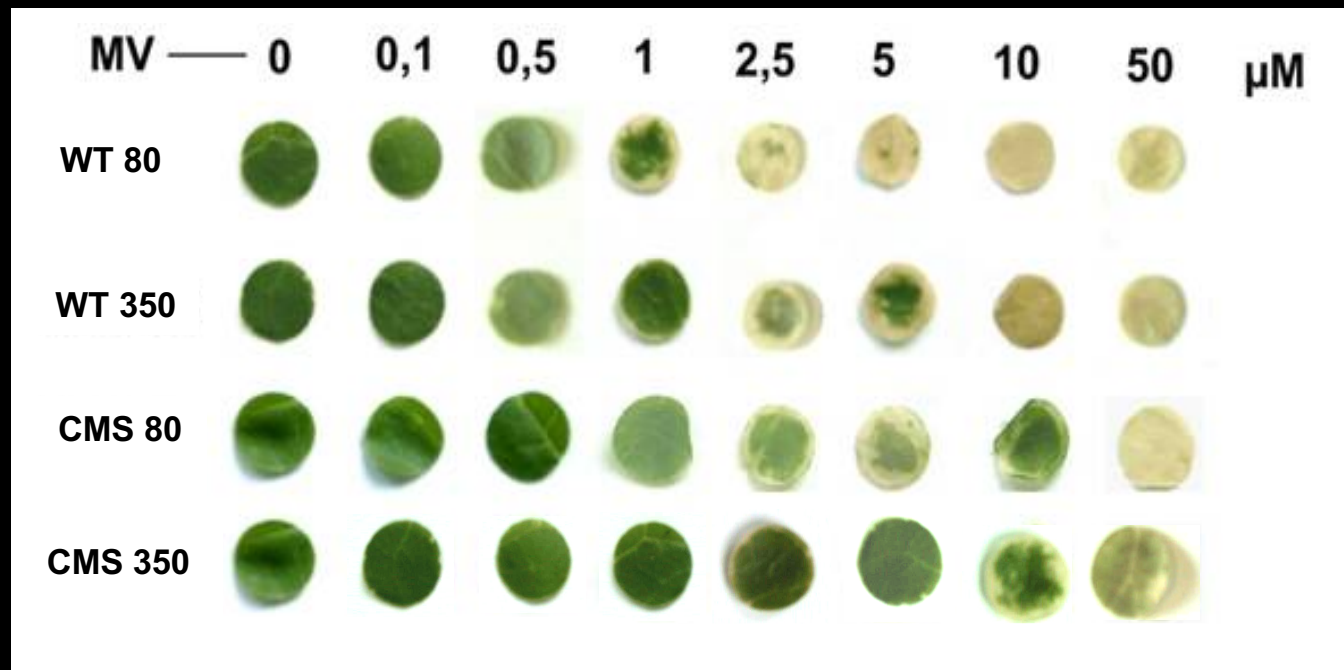
4. I^{ry} and II^{ry} reactions
of photosynthesis

3. Membrane integrity

STRESS RESPONSE

3.2 *Results: chlorophyll*

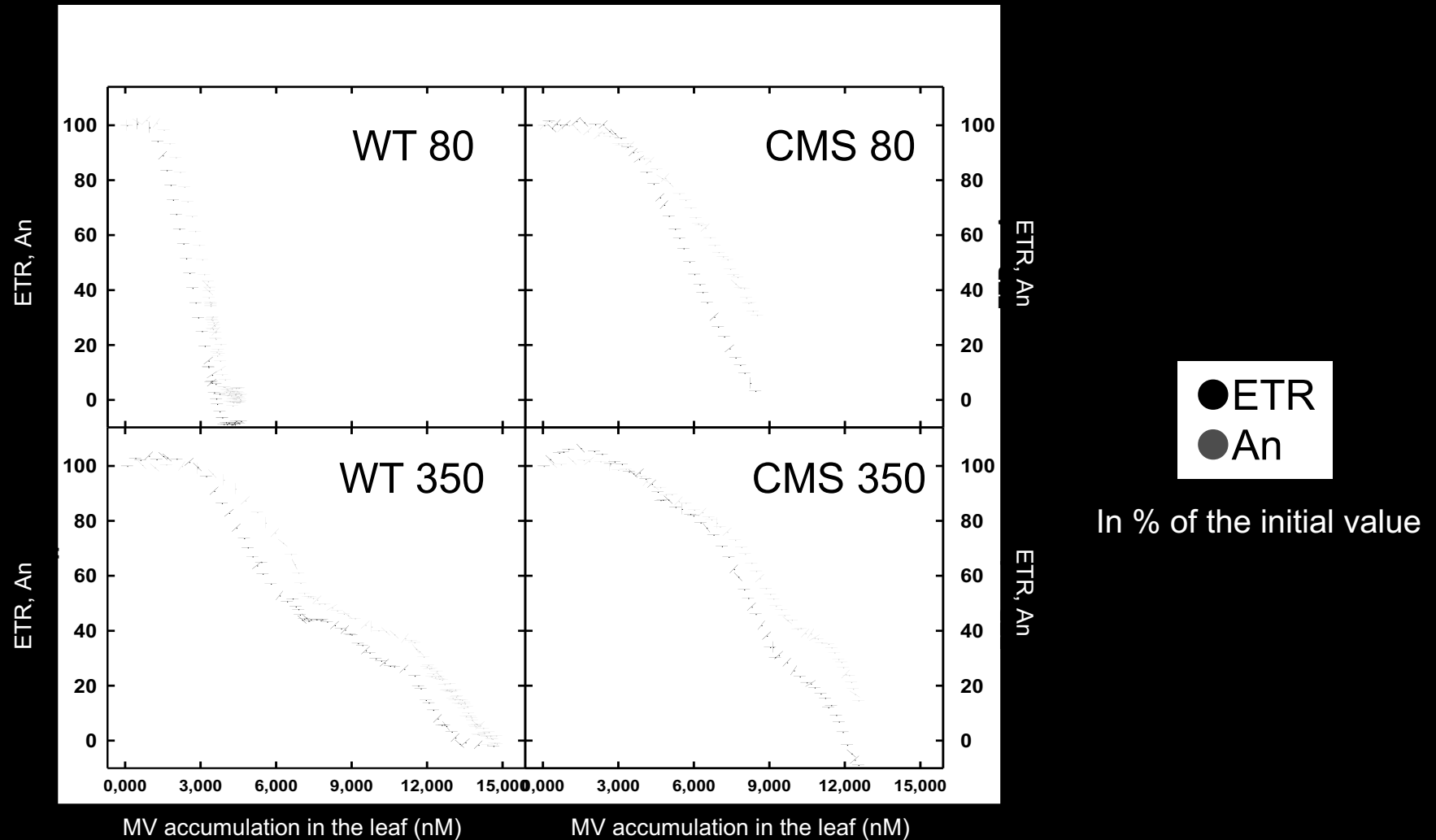
2. Leaf discs incubated 24h with different concentrations of MV under $1000 \mu\text{mol PAR m}^{-2} \text{s}^{-1}$.



STRESS RESPONSE

3.2 Results: A_n , ETR

4. Leaves incubated in 1mM MV under 1000 $\mu\text{mol PAR m}^{-2} \text{s}^{-1}$.



3.2 Questions

Carotenoids

Enzymes: CAT, SOD, APX

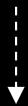
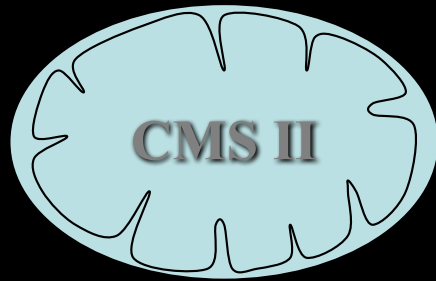
MV protection systems

Heat

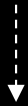
Conclusions

Taken together, these results show that **mitochondrial function is involved in:**

(1) abiotic stress responses



Altered gene expression



Higher detoxification system
+ Lower diffusion of MV



increased resistance to MV

Conclusions

Taken together, these results show that **mitochondrial function is involved in:**

(2) Photosynthetic acclimation

