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Mitochondria – Chloroplast interactions :

Acclimation of a *Nicotiana sylvestris* mitochondrial mutant to growth light intensity.



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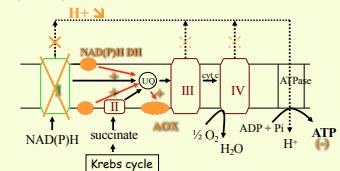
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The CMS II mutant of *Nicotiana sylvestris* is functionally defect in complex I of the mitochondrial electron transport chain (1) but has a slightly higher dark respiration rate. Furthermore, enzymes of the alternative respiratory pathway (NAD(P)H DH, AOX), are elevated in CMS as compared to the WT (see figure).

As mitochondrial function interacts with photosynthetic metabolism, this mutant was used as a tool to investigate the importance of mitochondria in photosynthetic acclimation to light.

Respiratory chain of CMS II mutant of *Nicotiana sylvestris*



• Spectrophotometric assays

RubisCO / NADP-MDH / SPS
Chl a/b

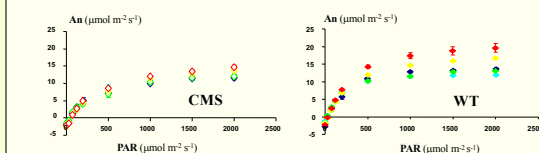
Acclimation to growth light intensity

• CO₂/H₂O exchanges Chl Fluorescence

(Licor 6400-40, Mini PAM, PAM 2000)

Are mutant leaves able to acclimate photosynthesis to growth light intensity ?

Investigations of light responses curves of carbon net assimilation in ambient air. The different colours represent a light growth intensity ranging from 80 (in bright blue) to 350 (in red) $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR.

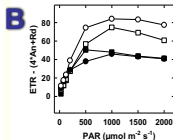
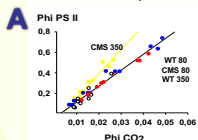


CMS leaves are unable to acclimate photosynthesis to higher growth light intensity as observed in the WT.

Further investigations compared low light grown CMS and WT leaves (LL=80 $\mu\text{mol m}^{-2} \text{s}^{-1}$: WT 80, CMS 80) with high light grown leaves (HL=350 $\mu\text{mol m}^{-2} \text{s}^{-1}$: WT 350 and CMS 350) only.

1 Are CMS leaves affected in primary photochemistry which might limit carbon assimilation ?

A: relation between quantum yields of PSII and of CO₂ assimilation calculated from light responses curves performed under 21% O₂ and 380 ppm CO₂. B: light response curve of excess electron transport not used for carbon assimilation calculated as ETR - (4*An+Rd) assuming that 4 electrons are necessary to assimilate one molecule of CO₂ (WT 80 : black squares ; CMS 80 : black circles ; WT 350 : white squares ; CMS 350 : white circles).



(A) Approximately 15 photons are used for the assimilation of one molecule of CO₂ in WT 80, WT 350 and CMS 80 but 23 photons are necessary in CMS 350. In an 0.5 % O₂ atmosphere, around 9 photons are necessary for the four types of plants.

(B) Excess ETR is higher in CMS 350 than in WT 350 requiring additional electrons sinks.

Priault et al., submitted (2)

2 Are CMS leaves affected in secondary photosynthetic reactions limiting carbon assimilation ?

Absolute and relative stimulation of initial Rubisco and SPS activities by high light (350 $\mu\text{mol m}^{-2} \text{s}^{-1}$) as compared to low light growth (80 $\mu\text{mol m}^{-2} \text{s}^{-1}$) in WT and CMS leaves measured either after one hour at growth light intensity or after 3h illumination at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR.

Rubisco			
growth light	$\mu\text{mol m}^{-2} \text{s}^{-1}$	13.9	3.3
	% stimulation	78	20
3h 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR	$\mu\text{mol m}^{-2} \text{s}^{-1}$	36.8	14.4
	% stimulation	183	58
SPS			
growth light	$\mu\text{mol m}^{-2} \text{s}^{-1}$	0.59	0.12
	% stimulation	155	20

• Stimulation of Rubisco and SPS initial activity by a higher growth light intensity is impaired in CMS.

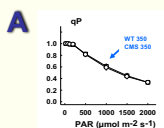
• Maximal activities are very similar between CMS 80 and WT 80 and increased in both genotypes in response to higher growth light intensity (data not shown).

Priault et al., submitted (2)

3 Is the acclimation response of CMS leaves affected by different redox homeostasis possibly regulating gene expression ?

Investigation of qP as redox parameter of the ETR chain and NADP-MDH as redox parameter of the chloroplast stroma.

A: Light response curve of the photochemical quenching (qP) reflecting the redox state of the plastoquinone pool (=1-qP). B: Initial and total activities of NADP-MDH ($\mu\text{mol m}^{-2} \text{s}^{-1}$) measured at growth light intensity after 1 h in the light period and after 3h illumination at 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PAR and 25 °C.



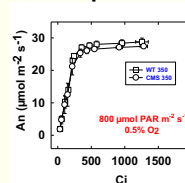
		WT 350		CMS 350	
		Initial	Total	Initial	Total
growth light	Initial	0.6 ± 0.05	0.6 ± 0.05	0.6 ± 0.05	0.6 ± 0.05
	Total	1.75 ± 0.07	1.46 ± 0.1	1.75 ± 0.07	1.46 ± 0.1
3h 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$	Initial	0.57 ± 0.06	0.65 ± 0.05	0.57 ± 0.06	0.65 ± 0.05
	Total	1.55 ± 0.12	1.38 ± 0.08	1.55 ± 0.12	1.38 ± 0.08

Priault et al., submitted (2)

• Redox parameters are not affected in CMS as compared to the WT.

4 The mismatch of ETR, carbon assimilation and initial activity of Rubisco is further investigated in CMS 350 leaves in order to characterize alternative electron sinks such as :

(a) Photorespiration :



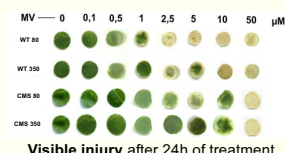
• Under non photorespiratory conditions, the difference between WT 350 and CMS 350 nearly disappears.

1. CMS and WT leaves have the same internal leaf CO₂ concentration (Ci) suggesting similar stomatal regulation.

2. Glycolate oxidase activity is about 15% higher in the CMS 350 than in the WT 350 after 1h of growth light.

3. WT 350 and CMS 350 have a similar specificity factor of Rubisco. (estimated from determination of I*)

4. In accordance with calculations from models, first experimental determinations of chloroplastic CO₂ concentration (Cc) by mass spectroscopy revealed a lower Cc in CMS 350 than in the WT 350.



Visible injury after 24h of treatment.

(b) oxidative metabolism :

Molecular biology and oxidative stress physiology are investigate to see whether excess electrons in the mutant induce higher oxidative stress tolerance.

• Chl content, Fv/Fm and Catalase activity are less affected by MV treatment in CMS plants than in WT plants independent of growth light intensity.

• The inhibition of electron transport and carbon net assimilation is delayed in CMS plants as compared to WT plants when incubated at the same concentration of MV.

These results show that mitochondrial function is necessary for acclimation of plants to growth light intensity and for oxidative stress response.

CMS plants do not acclimate to higher growth light intensity as the WT. This is shown by a lower quantum yield of CO₂ assimilation under ambient air conditions and lower activation state of photosynthetic enzymes. Currently, the fate of excess chloroplastic electrons in CMS plants grown at 350 $\mu\text{mol m}^{-2} \text{s}^{-1}$ as compared to the WT is investigated and tend to favour an higher photorespiration in the CMS 350.

CMS leaves showed higher resistance to MV-induced oxidative stress as indicated by lower: chlorophyll bleaching, photoinhibition of PS II and catalase activity. Furthermore, CMS leaves maintained higher rates of total chloroplastic electron transport and CO₂ assimilation after the same time of incubation and the same concentration of MV, suggesting that altered transcript and/or protein contents of some antioxidative enzymes (SOD, APX, GR and CAT) are involved in oxidative stress resistance, by conferring a more elevated protection in CMS than in the WT.

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References : (1) Gutierrez et al., 1997 (PNAS : 94, pp. 3436 - 3441) ; (2) P. Priault, C. Fresneau, G. Nector, R. DePaepe, G. Cornic and P. Streb The mitochondrial CMSII mutation of *Nicotiana sylvestris* impairs acclimation to growth irradiance (submitted to J. Exp. Bot.).