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Changes of alternative oxidase activity, capacity and protein content in leaves of *Cucumis sativus* wild-type and MSC16 mutant grown under different light intensities

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In vitro studies demonstrated that alternative oxidase (AOX) is biochemically regulated by a sulfhydryl-disulfide system, interaction with α-ketoacids, ubiquinone pool redox state and protein content among others. However, there is still scarce information about the in vivo regulation of the AOX. *Cucumis sativus* wild-type (WT) and MSC16 mutant plants were grown under two different light intensities and were used to analyze the relationship between the amount of leaf AOX protein and its in vivo capacity and activity at night and day periods. WT and MSC16 plants presented lower total respiration (V_t), cytochrome oxidase pathway (COP) activity (v_{cyt}) and alternative oxidase pathway (AOP) activity (v_{alt}) when grown at low light (LL), although growth light intensity did not change the amount of cytochrome oxidase (COX) nor AOX protein. Changes of v_{cyt} related to growing light conditions suggested a substrate availability and energy demand control. On the other hand, the total amount of AOX protein present in the tissue does not play a role in the regulation neither of the capacity nor of the activity of the AOP in vivo. Soluble carbohydrates were directly related to the activity of the AOP. However, although differences in soluble sugar contents mostly regulate the capacity of the AOP at different growth light intensities, additional regulatory mechanisms are necessary to fully explain the observed results.

Introduction

Plant mitochondria electron transport chain (ETC) is characterized by the existence of two electron transport pathways that branch at the ubiquinone (UQ) pool, the cytochrome oxidase pathway (COP) and the alternative oxidase pathway (AOP). The AOP is constituted by the alternative oxidase (AOX), a quinol-oxidizing protein that reduces oxygen to water. The electron transport through this pathway bypasses two of the three sites of proton extrusion from the COP, thus reducing the ATP synthesis from mitochondrial ETC (Moore and Siedow 1991). This energetically wasteful pathway has raised vast discussions about its biological function. To date, the

Abbreviations – AOP, alternative oxidase pathway; AOX, alternative oxidase; COP, cytochrome oxidase pathway; COX, cytochrome oxidase; HL, high light; LL, low light; SHAM, salicylhydroxamic acid; τ_a, electron partitioning through the alternative pathway; UQ, ubiquinone; v_{alt}, alternative oxidase pathway activity; V_{alt}, alternative oxidase pathway capacity; v_{cyt}, cytochrome oxidase pathway activity; V_t, total respiration.

only biological function attributed to AOP is related to the thermogenic flowering (Meeuse 1975, Watling et al. 2006). In non-thermogenic tissues, a general role for the AOP remains to be confirmed, and several hypotheses have been postulated. Among them, AOP is thought to sustain mitochondrial electron transport when there is high cell energy charge or COP restriction (Lambers et al. 2005), giving higher flexibility to respiratory metabolism under fluctuating environments. Under such conditions, AOP might also prevent over-reduction of the ETC reducing the formation of reactive oxygen species (Maxwell et al. 1999, Møller 2001).

The activity of the AOP can be regulated by the redox state and the size of the UQ pool (Dry et al. 1989, Ribas-Carbo et al. 1995b), post-translational modifications of AOX-like formation of inter-disulfide bonds (Umbach and Siedow 1993), protein content (Ribas-Carbo et al. 1997) and interaction with α-ketoacids (Millar et al. 1993). All these regulatory systems allow AOP to compete with an unsaturated COP which led to the conclusion that the only reliable method for measuring the activity of the AOP is the oxygen isotope fractionation technique (Day et al. 1996, Ribas-Carbo et al. 1995a). Using this technique, several studies have shown changes of the alternative pathway activity under phosphate starvation (Gonzalez-Meler et al. 2001), chilling recovery (Ribas-Carbo et al. 2000a), severe water stress (Ribas-Carbo et al. 2005a) during thermogenesis in flowers of the sacred lotus (Watling et al. 2006), after acclimation to elevated CO_2 (Gomez-Casanovas et al. 2007), with increased soil temperature (Rachmilevitch et al. 2007) and after induction of programmed cell death (Vidal et al. 2007).

Several studies have suggested that light might be involved in the regulation of AOP activity, and vice versa, i.e. AOP activity might be involved in the regulation of plant responses to changes in light intensity/quality. After transfer to high light (HL) conditions, the AOP and the type II NAD(P)H dehydrogenases have been considered to be efficient dissipation systems for excess reductants from chloroplast as a mechanism of plant photoprotection (Noguchi and Yoshida 2008). Moreover, AOP capacity has been related to the synthesis of ascorbic acid under photo-oxidative stress conditions (Bartoli et al. 2006). In addition, the electron partitioning between cytochrome and alternative pathways has recently been shown to be modified by phytochrome (Ribas-Carbo et al. 2008).

Earlier experiments showed higher activity of AOP in plants grown under HL (Noguchi et al. 2001). This increased AOP activity was related to higher levels of carbohydrates in sun species. Moreover, the AOP activity decreased from early to late night (Noguchi et al. 2001). On the other hand, in mitochondria isolated from leaves of *Cucumis sativus*, AOX protein and capacity decreased to undetectable levels after 8 h of darkness (Juszczuk et al. 2007). In leaves from these plants, AOX protein content was constitutively higher in MSC16, a genome rearrangement mutant that has lower Complex I activity, than in wild-type (WT) during the light period (Juszczuk and Rychter 2009) while no differences were observed in the in vivo activity of AOP (measured at dark) (Juszczuk et al. 2007). However, whether diurnal changes on AOX expression affect its in vivo activity remains to be determined.

Therefore, there seems to be some complex responses of AOP to diurnal and long-term variations in light intensities, probably involving both rapid and acclimation responses. In order to assess whether changes in AOX protein and AOP capacity affect light regulation of AOP, oxygen isotope fractionation during respiration and AOX protein content have been determined during day and night periods in leaves of *C. sativus* WT and MSC16 mutant grown under different light intensities (400 and 150 μmol photons m^{-2} s^{-1}).

Materials and methods

Plant material and growth conditions

Cucumber seeds (*C. sativus* L. cv. Borszczagowski) of WT or MSC16 mutant plants (obtained from Professor S. Malepszy, Warsaw Agricultural University) were germinated on wet filter paper, and after 4 days seedlings were transferred to 5 l pots containing a mixture of horticulture substrate and perlite (3:1). Plants were grown in a growth chamber under controlled conditions of 25/20°C day/night temperature, above 40% relative humidity and 16 h photoperiod, and were irrigated regularly with Hoagland solution. Two sets of plants were grown under two different light intensities: photosynthetic photon flux density (PPFD) of 400 μmol m^{-2} s^{-1}, considered HL, and 150 μmol m^{-2} s^{-1}, considered low light (LL). Leaf samples for all the biochemical and respiration measurements were taken from the first and the second fully expanded leaves after 4–6 h of the light period (day) or after 8–10 h dark period (night). WT and MSC16 plants used for sampling had developed four leaves. This stage was reached after 2 and 3 weeks after germination in HL grown WT and MSC16 plants and after 3 and 4 weeks after germination in LL grown WT and MSC16 plants, respectively.

AOP capacity measurements

Three 1 cm^2 leaf discs were harvested from the same leaf, weighted and incubated in a solution containing

30 m*M* MES pH 6.2, 0.2 m*M* $CaCl_2$ and potassium cyanide (KCN) 10 m*M* for 30 min in the dark. Oxygen uptake rates of the three leaf discs were measured in darkness using a liquid-phase Clark-type oxygen electrode (Rank Brothers) at a constant temperature of 25°C. The capacity of the AOP (V_{alt}) was determined as the cyanide resistant respiration that was sensitive to 20 m*M* salicylhydroxamic acid (SHAM). SHAM was prepared from a 1 *M* stock in dimethyl sulfoxide (DMSO). All solutions were always freshly prepared before use. To avoid oxygen-limiting conditions inside the cuvette, measurements were finished before oxygen concentration reached half air saturation levels. Five to eight replicates of three leaf discs each were performed at 8–10 h of dark period and at 4–6 h of light period from plants grown in LL or HL in four different days.

Respiration and oxygen isotope fractionation measurements

Before respiration analysis, leaves were placed in the dark for 30 min to avoid light-enhanced dark respiration. Then, one 10 cm^2 leaf disc was collected at 8–10 h of dark period and at 4–6 h of light period from plants grown in LL or HL in four different days. After harvesting, leaf discs were immediately weighted and placed in a 3 ml stainless-steel closed cuvette maintained at a constant temperature of 25°C using a copper plate and a serpentine around the cuvette with a temperature-controlled water bath (Florez-Sarasa et al. 2007).

Changes in the $^{18}O/^{16}O$ ratios and oxygen consumption were determined with a dual-inlet mass spectrometer system (Delta Plus XP, Thermo LCC, Bremen, Germany) as described in Florez-Sarasa et al. (2007). The mass ratios of 34/32 ($^{18}O_2/^{16}O_2$) and 32/28 ($^{16}O_2/^{28}N_2$) were obtained from standard and sample air with dual-inlet analysis, performing six replicate cycles for each respiration measurement. Calculations of the oxygen isotope fractionation were made as described in Ribas-Carbo et al. (2005b), and the electron partitioning between the two pathways in the absence of inhibitors was calculated as described by Guy et al. (1989). The r^2 values of all unconstrained linear regressions between – ln f and $\ln(R/R_o)$, with a minimum of five data points, were at least 0.995, considered minimally acceptable (Ribas-Carbo et al. 1997).

The electron partitioning through the AOP (τ_a) was calculated as follows:

$$\tau_a = \frac{\Delta_n - \Delta_c}{\Delta_a - \Delta_c}$$

where Δ_n, Δ_c and Δ_a are the oxygen isotope fractionation in the absence of inhibitors, in the presence of SHAM and in the presence of KCN, respectively. As COP discrimination has been shown to be very constant (Ribas-Carbo et al. 2005b), Δ_c value used was 20.0‰ obtained from Juszczuk et al. (2007). For KCN inhibitor treatments, leaf discs were incubated for 30 min by sandwiching between medical wipes soaked with a water solution of 10 m*M* KCN. In addition, a piece of medical wipe wetted with 10 m*M* KCN was placed in the cuvette. Δ_a value obtained was 31.3‰. All stock solutions were freshly prepared before use. The individual activities of the COP (v_{cyt}) and AOP (v_{alt}) were obtained by multiplying the total oxygen uptake rate (V_t) and the partitioning to each pathway as follows:

$$v_{cyt} = V_t \times (1-\tau_a)$$

$$v_{alt} = V_t \times \tau_a$$

Results are the average of four replicates with a different leaf disc each.

Extraction and determination of soluble protein and sugars

Three to four leaf samples (1.6 cm^2 leaf discs) were collected at 8–10 h of dark period and at 4–6 h of light period from plants grown in LL or HL in three to four different days and immediately frozen in liquid N_2. Leaf discs were ground to fine powder in the presence of polyvinylpolypyrrolidone (PVPP). Soluble proteins and sugars were extracted with a buffer containing 0.5 M Tris, 10 m*M* EDTA, 1% Triton X-100, 5 m*M* DTT and 0.25% protease-inhibitor cocktail (Sigma-Aldrich Co.). The homogenates were centrifuged at 15 000 g for 5 min at 4°C. Total soluble protein in the supernatant was determined according to Bradford (1976), and total soluble sugar was determined according to Roulin and Feller (2001).

Protein SDS–PAGE and immunodetection

Soluble fractions of the crude extracts were mixed 1:1 with SDS-sample buffer (0.5 M Tris, 0.3% Glycerol, 1.5% SDS, 0.15% Bromphenolblue and 5 μM DTT) and boiled for 5 min. One hundred micrograms of protein were loaded and separated on 12% SDS–PAGE gels and transferred to nitrocellulose membranes using wet Mini-PROTEAN system of Bio-Rad. A 1/50 dilution of the monoclonal antibody against AOX (Elthon et al. 1989), a 1/500 dilution of the monoclonal antibody against the voltage-dependent anion channel porin (PM035, from Dr Tom Elthon, Lincoln, NE), and a 1/3000 dilution of the polyclonal antibody against the subunit II of the cytochrome oxidase (COX) (AS04053A,

Table 1. Total respiration (V_t), electron partitioning through alternative pathway (τ_a), COP activity (v_{cyt}) and AOP activity (v_{alt}) in leaves of WT and MSC16 mutant *C. sativus* plants after 4–6 h light period (day) or after 8–10 h dark period (night) grown under HL and LL. Values are means and SE of four to eight replicates.

	HL				LL			
	Day		Night		Day		Night	
	WT	MSC16	WT	MSC16	WT	MSC16	WT	MSC16
V_t	1.58 ± 0.18	1.68 ± 0.13	1.66 ± 0.08	1.78 ± 0.19	0.91 ± 0.09	1.14 ± 0.06	1.06 ± 0.03	1.10 ± 0.06
τ_a	0.28 ± 0.02	0.31 ± 0.01	0.25 ± 0.01	0.31 ± 0.01	0.29 ± 0.02	0.30 ± 0.01	0.27 ± 0.02	0.30 ± 0.00
v_{cyt}	1.14 ± 0.16	1.16 ± 0.09	1.24 ± 0.06	1.22 ± 0.12	0.65 ± 0.08	0.80 ± 0.05	0.77 ± 0.05	0.77 ± 0.05
v_{alt}	0.44 ± 0.03	0.52 ± 0.05	0.42 ± 0.03	0.56 ± 0.07	0.26 ± 0.01	0.34 ± 0.01	0.29 ± 0.01	0.33 ± 0.02
V_{alt}	1.54 ± 0.14	1.39 ± 0.17	1.29 ± 0.08	1.32 ± 0.07	1.03 ± 0.06	1.03 ± 0.08	1.00 ± 0.04	1.06 ± 0.10

Agrisera Co., Sweden) were used as primary antibodies. Immunodetection of mitochondrial proteins (AOX, COX and porin) via colorimetric assay was carried out with the BCIP/NBT alkaline phosphatase system according to the manufacturer's instructions (Sigma-Aldrich Co.). Densitometry quantification of the bands was made with TotalLab Software (Nonlinear Dynamics Ltd, UK).

Statistical analyses

A multivariant analysis of variance (ANOVA) with a level of significance of *P*-value <0.05 was performed with SPSS for Windows 16.0. Duncan post hoc test was used when statistically significant differences were obtained.

Results

Leaf respiration, oxygen isotope fractionation, mitochondrial oxidases protein amount and soluble sugar contents were measured, after 4–6 h of light and after 8–10 h of darkness, in leaves of WT and MSC16 *C. sativus* L. cv. Borszczagowski grown at light intensities of 400 µmol m^{-2} s^{-1} (HL) and 150 µmol m^{-2} s^{-1} (LL).

Effect of growing light intensity on respiratory activities, protein content and soluble carbohydrate amounts

Higher growth light intensity induced higher total respiration (V_t), COP activity (v_{cyt}), AOP activity (v_{alt}) and AOP capacity (V_{alt}) in WT and MSC16 regardless of the time of measurement (Tables 1 and 2). The electron partitioning through AOX (τ_a) remained constant (Table 1), and hence the COX/AOX proportion was maintained in both WT and MSC16 leaves. Unlike respiration rates, growth light intensity did not change COX and AOX protein content in both WT and MSC16 (Fig. 1). Soluble sugar amounts were significantly higher in HL-grown than in LL-grown plants (Table 2 and Fig. 2).

Table 2. Multivariant ANOVA analysis of all the variables measured. Plant type, growth light and measurement time were analyzed as independent factors. The double and triple interactions were omitted as they were not significant with a *P*-value of <0.05. Asterisks indicate a *P*-value of <0.05.

	Growth light	Plant type	Measurement time
V_t	0.000*	0.087	0.230
τ_a	0.622	0.001*	0.075
v_{cyt}	0.000*	0.487	0.187
v_{alt}	0.000*	0.000*	0.531
V_{alt}	0.052	0.940	0.636
AOX/porin	0.223	0.037*	0.335
Soluble carbohydrates	0.001*	0.006*	0.242

Comparison of WT and MSC16 plants

Total respiration, v_{cyt} and V_{alt} were similar between WT and MSC16 within any given treatment (Tables 1 and 2). τ_a and v_{alt} were significantly higher in MSC16 than in WT (Tables 1 and 2). Similarly, AOX protein content was significantly higher (Fig. 1 and Table 2) in MSC16 than in WT while COX protein levels were similar (Fig. 1). Moreover, soluble carbohydrate contents were higher in MSC16 than in WT (Table 2 and Fig. 2).

Comparison of day and night measurements

In general, V_t, v_{cyt}, v_{alt} and V_{alt} showed no significant differences between day and night (Tables 1 and 2). In addition, no changes in protein content of both COX and AOX were observed between day and night (Fig. 1 and Table 2). Similarly, total soluble carbohydrate content was not significantly different between day and night periods (Fig. 2 and Table 2).

Discussion

The initial goal of this research was to study how the electron partitioning between mitochondrial respiratory

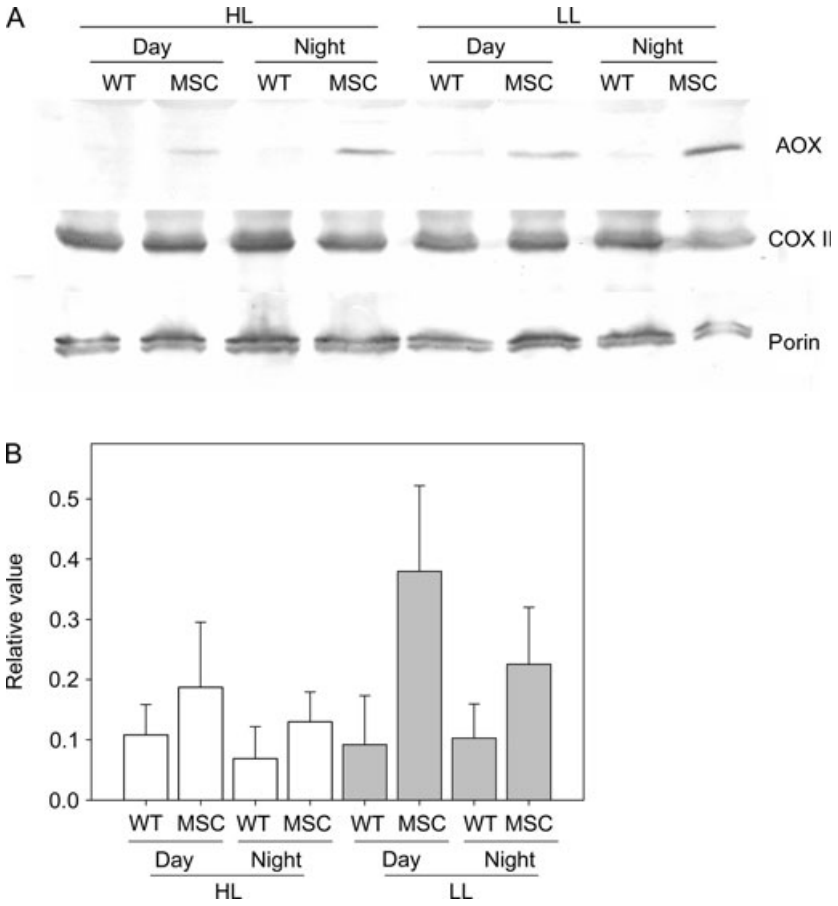

Fig. 1. (**A**) Western blot analysis of AOX, COX and mitochondrial porin proteins in leaves of WT and MSC16 mutant (MSC) *C. sativus* plants after 4–6 h of the light period (day) or after 8–10 h dark period (night) grown under HL and LL. (**B**) Relative values of AOX protein abundance were obtained by dividing densitometry of AOX and porin bands of the western blots. White and gray bars indicate HL and LL grown plants, respectively. Values are means and SE of three replicates.

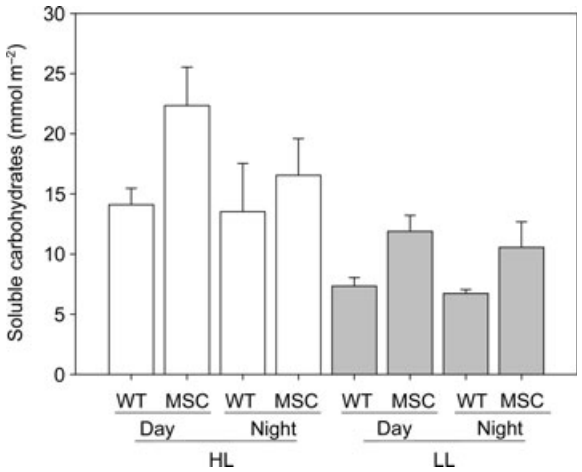

Fig. 2. Content of total soluble carbohydrates of leaves of WT and MSC16 mutant (MSC) *C. sativus* plants after 4–6 h light period (day) or after 8–10 h dark period (night) grown under HL (white bars) and LL (gray bars). Values are means and SE of three to four replicates.

pathways behaves in leaves from plants grown under different light intensities. Considering that the regulation of the activity of the AOX was proposed to be related to the amount of AOX protein present, leaves of *C. sativus* WT and MSC16 were selected because they present different amounts of AOX protein (Juszczuk et al. 2007).

In agreement with the results of Noguchi et al. (2001), respiration was lower in plants grown under LL, both in WT and MSC16, concomitant with lower soluble carbohydrates content. Consequently, it is quite likely that the lower availability of sugars limits the activity of the cytochrome pathway (v_{cyt}) in WT plants grown under LL. Moreover, Szal et al. (2008) observed that during light, NAD(H) mitochondrial fraction of MSC16 was more oxidized compared with WT which could explain why although MSC16 leaves showed higher levels of soluble carbohydrates than WT leaves (Fig. 2) they presented similar v_{cyt} (Table 1). Higher levels of soluble carbohydrates in MSC16 leaves suggest that

these plants have a limited carbohydrate export from leaves, since the production of sugars must be lower and its consumption higher than in WT, due to slightly lower photosynthesis and slightly higher respiration rates, respectively (Juszczuk et al. 2007). A limitation in the export of sugars may partially explain why growth of these plants is severely restricted when compared with WT, and thus it deserves further experiments. Regardless of the mechanisms, the higher amounts of soluble sugars in leaves of MSC16 plants imply that additional control of v_{cyt} may be present. This has previously been observed in roots of *Poa annua*, where soluble carbohydrates were not the only point of control of v_{cyt} (Millenaar et al. 2002), and in leaves of *Nigella sativa* and *Proteus vulgaris*, where v_{cyt} was suggested to be regulated by carbohydrates, Pi and ADP availability (Gonzalez-Meler et al. 2001). Notably, no major changes were observed on the COX protein content in all conditions tested in both WT and MSC16 (Fig. 1). Taken together, these results suggest that soluble sugars might be important in controlling COX activity in *C. sativus* leaves (Fig. 3), although control by ATP demand due to reduced costs for maintenance of photosynthetic components and carbohydrates export under LL conditions (Noguchi 2005) cannot be discarded.

Regarding the AOP, a clear relationship between the amount of protein of AOX and the capacity of the AOP (V_{alt}) has previously been described (Lennon et al. 1997, Gonzalez-Meler et al. 1999). However, in the present experiments, V_{alt} (Table 1) did not correlate with the amount of protein (Fig. 1). This would indicate that an additional regulation of V_{alt} was present. Other regulations besides protein content might be substrate limitation or post-translational control. The fact that V_{alt} was similar to V_t under LL conditions, in both WT and MSC16 leaves, would indicate that V_{alt} might be underestimated due to substrate limitation, as discussed in McDonald et al. (2002). On the other hand, similar V_{alt} in WT and MSC16 leaves, despite higher content of soluble carbohydrates and AOX protein content, would suggest the presence of an additional post-translational control of AOX protein.

In isolated mitochondria from *C. sativus*, Juszczuk et al. (2007) observed the disappearance of AOX protein after 8 h of darkness. Unlike in isolated mitochondria, the amount of AOX protein present in leaves does not significantly change between day and night measurements (Fig. 1). This reinforces previous observations indicating that the AOX gene transcripts seem to be diurnally regulated, but post-translational regulation of AOX activity might be energetically more favored than daily protein degradation and resynthesis (Rasmusson and Escobar 2007).

AOX activity (v_{alt}) in shade species has been suggested to be regulated by the reduction state of its disulfide bonds under LL conditions (Noguchi et al. 2005). Moreover, the UQ pool could be less reduced due to the lower level of respiratory substrates (Noguchi et al. 2001). This would explain the lower AOX activity observed in both WT and MSC16 leaves (Table 1). Similar results have been obtained in *Arabidopsis thaliana* leaves where v_{alt} was lower under LL compared with HL growing conditions. However, when plants have been subjected to continuous darkness and depletion of carbohydrates, no major changes in v_{alt} have been observed (I. Florez-Sarasa, unpublished results) similar to the observations in roots of *P. annua* after transfer from HL to LL conditions (Millenaar et al. 2000).

A clear relationship between AOX activity and soluble carbohydrate content has been shown here (Fig. 3). Regulation of v_{alt} in leaves of different species has been shown to be independent of the level of carbohydrates (Gonzalez-Meler et al. 2001) and the regulation of v_{alt} by light was not related to carbohydrates content (Ribas-Carbo et al. 2000b), however, in our study, a direct effect of light intensity cannot be separated of carbohydrate content, as both have been decreased in LL growing conditions. Nevertheless, higher v_{alt} in MSC16 leaves with respect to WT leaves could be explained by higher levels of respiratory substrates, because soluble sugar contents were higher in MSC16 than in WT regardless of the growing light intensity.

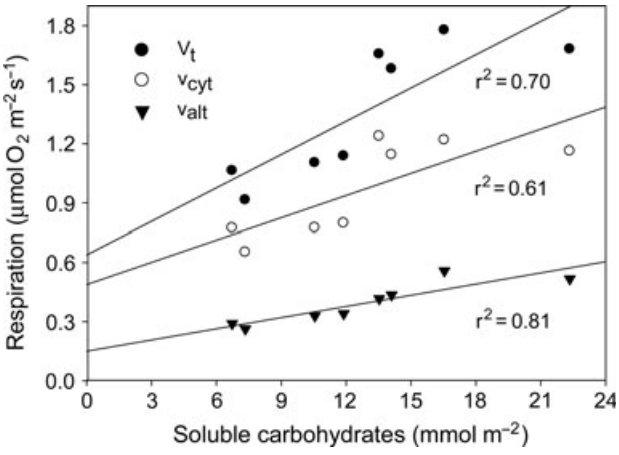

Fig. 3. The relationship between respiratory activities (total respiration [V_t], COP activity [v_{cyt}] and AOP activity [v_{alt}]) and total soluble carbohydrates in leaves of WT and MSC16 mutant (MSC) *C. sativus* plants after 4–6 h light period (day) or after 8–10 h dark period (night) grown under HL and LL. Correlation coefficients are indicated as r^2 values.

Concluding remarks

Changes in growth light intensity induce changes in the activity of the alternative pathway in leaves

The total amount of AOX protein present in the tissue does not play a role in the regulation neither of the capacity nor of the activity of the alternative pathway in vivo. Although carbohydrates are directly related with the activity of the alternative pathway, additional effects of the growth light intensity cannot be discarded as both have not been separated. This is of special importance because the capacity of the alternative pathway is clearly not only regulated by soluble sugar content.

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References

Bartoli G, Yu J, Gomez F, Fernandez L, McIntosh L, Foyer CH (2006) Inter-relationships between light and respiration in the control of ascorbic acid synthesis and accumulation in Arabidopsis thaliana leaves. Journal of Experimental Botany 57: 1621–1631

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254

Day DA, Krab K, Lambers H, Moore AL, Siedow JN, Wagner AM, Wiskich JT (1996) The cyanide-resistant oxidase: to inhibit or not to inhibit, that is the question. Plant Physiol 110: 1–2

Dry IB, Moore AL, Day DA, Wiskich JT (1989) Regulation of alternative pathway activity in plant mitochondria: nonlinear relationship between electron flux and the redox poise of the quinone pool. Arch Biochem Biophys 273: 148–157

Elthon TE, Nickels RL, McIntosh L (1989) Monoclonal antibodies to the alternative oxidase of higher plant mitochondria. Plant Physiol 89: 1311–1317

Florez-Sarasa ID, Bouma TJ, Medrano H, Azcon-Bieto J, Ribas-Carbo M (2007) Contribution of the cytochrome and alternative pathways to growth respiration and maintenance respiration in *Arabidopsis thaliana*. Physiol Plant 129: 143–151

Gomez-Casanovas N, Blanc-Betes E, Gonzalez-Meler MA, Azcon-Bieto J (2007) Changes in respiratory mitochondrial machinery and cytochrome and alternative pathway activities in response to energy demand underlie the acclimation of respiration to elevated CO_2 in the invasive *Opuntia ficus-indica*. Plant Physiol 145: 49–61

Gonzalez-Meler MA, Ribas-Carbo M, Giles L, Siedow JN (1999) The effect of growth and measurement temperature on the activity of the alternative respiratory pathway. Plant Physiol 120: 765–772

Gonzalez-Meler MA, Giles L, Thomas RB, Siedow JN (2001) Metabolic regulation of leaf respiration and alternative pathway activity in response to phosphate supply. Plant Cell Environ 24: 205–215

Guy RD, Berry JA, Fogel ML, Hoering TC (1989) Differential fractionation of oxygen isotopes by cyanide-resistant and cyanide-sensitive respiration in plants. Planta 177: 483–491

Juszczuk IM, Rychter AM (2009) BN-PAGE analysis of the respiratory chain complexes in mitochondria of cucumber MSC16 mutant. Plant Physiology and Biochemistry 47: 397–406

Juszczuk IM, Flexas J, Szal B, Dabrowska Z, Ribas-Carbo M, Rychter AM (2007) Effect of mitochondrial genome rearrangement on respiratory activity, photosynthesis, photorespiration and energy status of MSC16 cucumber (*Cucumis sativus*) mutant. Physiol Plant 131: 527–541

Lambers H, Robinson SA, Ribas-Carbo M (2005) Regulation of respiration in vivo. In Lambers H, Ribas-Carbo M (eds) Plant Respiration: From Cell to Ecosystem Vol. 18. Advances in Photosynthesis and Respiration Series. Springer, Dordrecht, The Netherlands, pp 1–15

Lennon AM, Neuenschwander UH, Ribas-Carbo M, Giles L, Ryals JA, Siedow JN (1997) The effects of salicylic acid and tobacco mosaic virus infection on the alternative oxidase of tobacco. Plant Physiol 115: 783–791

Maxwell DP, Wang Y, McIntosh L (1999) The alternative oxidase lowers mitochondrial reactive oxygen production in plant cells. Proc Natl Acad Sci USA 96: 8271–8276

McDonald AE, Sieger SM, Vanlerberghe GC (2002) Methods and approaches to study plant mitochondrial alternative oxidase. Physiol Plant 116: 135–143

Meeuse BDJ (1975) Thermogenic respiration in aroids. Annu Rev Plant Physiol 26: 117–126

Millar AH, Wiskich JT, Whelan J, Day DA (1993) Organic acid activation of the alternative oxidase of plant mitochondria. FEBS Lett 329: 259–262
Millenaar FF, Roelofs R, Gonzalez-Meler MA, Siedow JN, Wagner AM, Lambers H (2000) The alternative oxidase in roots of *Poa annua* after transfer from high-light to low-light conditions. Plant J 23: 623–632
Millenaar FF, Gonzalez-Meler MA, Siedow JN, Wagner AM, Lambers H (2002) Role of sugars and organic acids in regulating the concentration and activity of the alternative oxidase in *Poa annua* roots. J Exp Bot 53: 1081–1088
Møller IM (2001) Plant mitochondria and oxidative stress: electron transport, NADH turnover, and metabolism of reactive oxygen species. Annu Rev Plant Physiol Plant Mol Biol 52: 561–591
Moore AL, Siedow JN (1991) The regulation and nature of the cyanide-resistant alternative oxidase of plant mitochondria. Biochim Biophys Acta 1059: 121–140
Noguchi K (2005) Effects of light intensity and carbohydrate status on leaf and root respiration. In Lambers H, Ribas-Carbo M (eds) Plant Respiration: From Cell to Ecosystem Vol. 18. Advances in Photosynthesis and Respiration Series. Springer, Dordrecht, The Netherlands, pp 63–83
Noguchi K, Yoshida K (2008) Interaction between photosynthesis and respiration in illuminated leaves. Mitochondrion 8: 87–99
Noguchi K, Go CS, Terashima I, Ueda S, Yoshinari T (2001) Activities of the cyanide-resistant respiratory pathway in leaves of sun and shade species. Aust J Plant Physiol 28: 27–35
Noguchi K, Taylor NL, Millar AH, Lambers H, Day DA (2005) Response of mitochondria to light intensity in the leaves of sun and shade species. Plant Cell Environ 28: 760–771
Rachmilevitch R, Xu Y, Gonzalez-Meler MA, Huang BR, Lambers H (2007) Cytochrome and alternative pathway activity in roots of thermal and non-thermal Agrostis species in response to high soil temperature. Physiol Plant 129: 163–174
Rasmusson AG, Escobar MA (2007) Light and diurnal regulation of plant respiratory gene expression. Physiol Plant 129: 57–67
Ribas-Carbo M, Berry JA, Yakir D, Giles L, Robinson SA, Lennon AM, Siedow JN (1995a) Electron partitioning between the cytochrome and alternative pathways in plant mitochondria. Plant Physiol 109: 829–837
Ribas-Carbo M, Wiskich JT, Berry JA, Siedow JN (1995b) Ubiquinone redox behaviour in plant mitochondria during electron transport. Arch Biochem Biophys 317: 156–160
Ribas-Carbo M, Lennon AM, Robinson SA, Giles L, Berry JA, Siedow JN (1997) The regulation of electron partitioning between the cytochrome and alternative pathways in soybean cotyledon and root mitochondria. Plant Physiol 113: 903–911
Ribas-Carbo M, Aroca R, Gonzalez-Meler MA, Irigoyen JJ, Sanchez-Diaz M (2000a) The electron partitioning between the cytochrome and alternative pathways during chilling recovery in two cultivars differing in chilling sensitivity. Plant Physiol 109: 829–837
Ribas-Carbo M., Robinson SA, Gonzalez-Meler MA, Lennon AM, Giles L, Siedow JN, Berry JA (2000b) Effects of light on respiration and oxygen isotope fractionation in soybean cotyledons. Plant Cell Environ 23: 983–989
Ribas-Carbo M, Taylor NL, Giles L, Busquets S, Finnegan PM, Day DA, Lambers H, Medrano H, Berry JA, Flexas J (2005a) Effects of water stress on respiration in soybean leaves. Plant Physiol 139: 466–473
Ribas-Carbo M, Robinson SA, Giles L (2005b) The application of the oxygen-isotope technique to assess respiratory pathway partitioning. In Lambers H, Ribas-Carbo M (eds) Plant Respiration: From Cell to Ecosystem Vol. 18. Advances in Photosynthesis and Respiration Series. Springer, Dordrecht, The Netherlands, pp 31–42
Ribas-Carbo M, Giles L, Flexas J, Briggs W, Berry JA (2008) Phytochrome driven changes in respiratory electron transport partitioning in soybean (*Glycine max* L.) cotyledons. Plant Biol 10: 281–287
Roulin F, Feller U (2001) Reversible accumulation of (1–3, 1–4)-β-glucan endohydrolase in wheat leaves under sugar depletion. J Exp Bot 52: 2323–2332
Szal B, Dabrowska S, Malmberg G, Gardeström P, Rychter AM (2008) Changes in energy status of leaf cells as a consequence of mitochondrial genome rearrangement. Planta 227: 697–706
Umbach AL, Siedow JN (1993) Covalent and noncovalent dimers of the cyanide-resistant oxidase protein in higher plant mitochondria and their relationship to enzyme activity. Plant Physiol 103: 845–854
Vidal G, Ribas-Carbo M, Garmier M, Dubertret G, Rasmusson AG, Mathieu C, Foyer CH, De Paepe R (2007) Lack of respiratory chain complex I impairs alternative oxidase engagement and modulates redox signaling during elicitor-induced cell death in tobacco. Plant Cell 19: 640–655
Watling JR, Robinson SA, Seymour RS (2006) Contribution of the alternative pathway respiration during thermogenesis in flowers of the sacred lotus. Plant Physiol 140: 1367–1373

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