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ORIGINAL ARTICLE

Organ specific analysis of the anaerobic primary metabolism in rice and wheat seedlings II: Light exposure reduces needs for fermentation and extends survival during anaerobiosis

Angelika Mustroph · Elena I. Boamfa · Lucas J. J. Laarhoven · Frans J. M. Harren · Yvonne Pörs · Bernhard Grimm

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Abstract Low oxygen stress in plants can occur during flooding and compromise the availability and utilization of carbohydrates in root and shoot tissues. Low-oxygen-tolerant rice and -sensitive wheat plants were analyzed under anaerobiosis in light to evaluate main factors of the primary metabolism that affect sensitivity against oxygen deprivation: activity of glycolysis and the rate of photosynthesis. Relatively stable ATP contents (93 and 58% of aerated control levels after 24 h anaerobiosis) in illuminated shoot tissues account for enhanced tolerance of rice and wheat seedlings to anaerobiosis upon light exposure in comparison to anoxia in darkness. Although the photosynthetic process was inhibited during low oxygen stress, which was partly due to CO_2 deficiency, more light-exposed than dark-incubated seedlings survived. Illuminated plants could tolerate a 70% lower anaerobic ethanol production in shoots in comparison to darkness, although still an 18-times higher ethanol production rate was determined in rice than in wheat leaves. In conclusion, light-exposed plants grown under anaerobiosis may recycle low amounts of generated oxygen between photosynthesis and dissimilation and generate additional energy not only from substrate phosphorylation during glycolysis but also from other sources like cyclic electron transport.

Keywords Anaerobiosis · Chlorophyll fluorescence · Cyclic electron transport · Fermentation · *Oryza* · *Triticum*

Abbreviations

ADH	Alcohol dehydrogenase
AEC	Adenylate energy charge
Chl	Chlorophyll
DEPS	De-epoxidation state of xanthophylls
ETR	Electron transport rate
$J_{CO_{2max}}$	CO_2 Uptake rate at saturating PPFD
NDH	NAD(P)H PQ Oxidoreductase complex
PDC	Pyruvate decarboxylase
PPFD	Photosynthetic photon flux density
PQ	Plastoquinone
PS	Photosystem
R_D	Dark respiration rate
$\Phi_{CO_{2app}}$	Apparent quantum yield of CO_2 uptake
Φ_{max}	Maximum quantum yield of PSII photochemistry

A. Mustroph (✉) · Y. Pörs · B. Grimm
Department of Plant Physiology,
Humboldt-University Berlin, Philippstrasse 13/Haus 12,
10115 Berlin, Germany
e-mail: angelika.mustroph@web.de

E. I. Boamfa · L. J. J. Laarhoven · F. J. M. Harren
Life Science Trace Gas Exchange Facility,
Molecular and Laser Physics, Radboud University,
Toernooiveld 1, 6525 Nijmegen, The Netherlands

Y. Pörs
Department of Cell Biology, Humboldt-University Berlin,
Invalidenstrasse 42, 10115 Berlin, Germany

Introduction

Rice (*Oryza sativa* L.) and wheat (*Triticum aestivum* L.) significantly differ in their tolerance to low oxygen stress. While rice is able to germinate without oxygen

and to withstand several days of low oxygen stress, wheat seedlings cannot germinate under anaerobiosis and die much earlier (Menegus et al. 1991; Guglielminetti et al. 1997; Mustroph and Albrecht 2003). One of several reasons for the diversity of tolerance seems to be the capacity of the plants to consume carbohydrates for ethanolic fermentation during the anaerobic period. In the first part of our comprehensive analysis on different activities of the primary metabolism in wheat and rice under low oxygen stress, we observed that during 20 h of anaerobiosis in darkness, rice released 14 times more ethanol than wheat. The ethanol emission correlated with a longer anaerobic resistance of rice than of wheat plants, as well as with higher sugar supply and higher glycolytic and fermentative enzyme activities in rice shoots. However, even the tolerant rice seedlings could not survive more than 24 h of anaerobiosis in the dark (Mustroph et al. 2006).

Previously, it was demonstrated that light during the anaerobic incubation enhances the survival rate of rice seedlings compared to their continuous dark incubation (Mustroph and Albrecht 2003). Other studies have shown that clear floodwater increases the survival rate of submerged rice plants in comparison to muddy water (Ram et al. 2002). However, the molecular reasons for an enhanced survival rate during oxygen deficiency and light exposure were not sufficiently examined. When plants are exposed to light during low oxygen stress, it is assumed that photosynthesis enables formation of carbohydrates and oxygen, which are consumed during glycolysis and respiration (Mommer and Visser 2005; Rolletschek et al. 2005).

Studies about photosynthetic processes in shoots subjected to anaerobiosis are rare and were mainly performed during the re-aeration period (Schlüter and Crawford 2001, 2003). In the past in most experiments on photosynthesis upon low oxygen stress, plants were exposed to flooding stress, and it was observed that an impairment of photosynthesis followed root death and stomata closure (Liao and Lin 1996; Ladygin and Semenova 1999; Holmer and Bondgaard 2001; Kawano et al. 2002).

We examined dissimilation and fermentation as well as photosynthetic processes in flooding-tolerant rice and -sensitive wheat seedlings to find out to which extent plants benefit from light-dependent processes during anaerobiosis. The experiments were performed in a nitrogen atmosphere, which allowed comparison with experiments in anaerobiosis during dark incubation (Mustroph et al. 2006). Apart from on-line monitoring of the released fermentative products acetaldehyde and ethanol by a laser-based photoacoustic trace gas detection system (Boamfa et al. 2003), several photosynthetic and biochemical parameters were analyzed during and after the exposure of rice and wheat seedlings to anaerobic stress in light.

Materials and methods

Germination and plant culture

Caryopses of rice (*O. sativa* cv. Cigalon, Union des Coopératives Agricoles de Semences de Provence, Arles, France) and wheat (*T. aestivum* cv. Alcedo, SW Seed Hadmersleben GmbH, Hadmersleben, Germany) were germinated in the dark at 25°C on moist filter paper and were transferred after 2 days to an aerated hydroponic KNOP nutrient solution [5 mM $Ca(NO_3)_2{\cdot}4H_2O$, 1.6 mM KCl, 1.8 mM KH_2PO_4, 1 mM $MgSO_4{\cdot}7H_2O$, 0.7 mM Fe-Na-EDTA, 0.15 μM KJ, 0.6 μM LiCl, 0.2 μM $CuSO_4{\cdot}5H_2O$, 0.35 μM $ZnSO_4{\cdot}7H_2O$, 0.8 μM H_3BO_3, 0.15 μM $Al_2(SO_4)_3$, 1.77 μM $MnCl_2{\cdot}4H_2O$, 0.19 μM $NiSO_4{\cdot}6H_2O$, 0.17 μM $Co(NO_3)_2{\cdot}6H_2O$, 0.21 μM KBr, 0.21 μM $(NH_4)_6Mo_7O_{24}$]. The plants were grown in an environmental chamber (MLR-350, Sanyo, Osaka, Japan), under a 16 h light/8 h dark regime of 22/17°C with photosynthetic photon flux density (PPFD) of 300 μmol m^{-2} s^{-1}. Fourteen-day-old rice and 9-day-old wheat plants were used for measurements.

For analyses of metabolites and enzyme activities, plants were treated as follows. Seedlings were placed in a desiccator, which was continuously bubbled with nitrogen gas, under normal day–night regime. Anoxic treatment was initiated 6 h after start of illumination. The exact time course for this treatment is shown in Fig. 2c, d. After 4 and 24 h stress, plants were harvested, frozen immediately in liquid N_2, and stored at –80°C until extraction.

Detection of acetaldehyde, ethanol, CO_2, and oxygen release

Acetaldehyde, ethanol, and CO_2 release were detected as described in Mustroph et al. (2006). Anaerobic and aerobic gas phase conditions were imposed on rice and wheat seedlings, using flows of nitrogen gas and air under different light regimes. Experiments were made in a two-compartment glass cuvette and a whole-plant cuvette as described in Mustroph et al. (2006). Illumination was applied with an intensity of 350 μmol m^{-2} s^{-1}. The lamps warmed up the cuvette to 26°C during illumination.

Detection of carbohydrate and pigment contents, and enzyme activities

Glucose, fructose, sucrose, starch, ethanol, and adenylate contents as well as Alcohol dehydrogenase (ADH) and Pyruvate decarboxylase (PDC) activities in root and shoot tissues were extracted and measured as described in Mustroph et al. (2006). The adenylate energy charge (AEC) was calculated in accordance to Atkinson (1968) as AEC=(ATP+0.5ADP)/(ATP+ADP+AMP).

Chlorophylls and xanthophylls were extracted from leaves and detected by HPLC analysis modified after Thayer and Bjoerkman (1990) and Woitke et al. (1994). Frozen tissue (200 mg) was ground in liquid nitrogen, a spatula tip of $MgCO_3$ was added, and the material was homogenized in 1 ml 85% acetone. After centrifugation at 4°C and 13,000×*g*, the pellet was re-extracted with 600 µl 100% acetone. Both supernatants were mixed and used for detection of pigments. Pigments of the extracts were separated by HPLC (Agilent 1100 Series, Agilent, Böblingen, Germany) using an RP 18 column with a flow rate of 1 ml min^{-1}. The gradient was accomplished with acetonitrile–water–triethylamine (180:20:0.2) (solution A) and ethylacetate (solution B). Pigments were identified and quantified using a diode array detector (Agilent 1100 Series). Standards were used for calibration. The de-epoxidation state of xanthophyll-cycle-pigments (DEPS) was calculated as DEPS = [(Zeaxanthin + 0.5 Antheraxanthin)/(Violaxanthin + Antheraxanthin + Zeaxanthin), Demming-Adams and Adams 1996].

CO_2 gas exchange

Gas exchange measurements were carried out on attached leaves in an open CO_2/H_2O gas exchange cuvette system using an infrared gas analyzer (LI–6400, Fa. LI-COR Biosciences Inc., Lincoln, NE, USA). Recordings were taken at a temperature of 22°C in the leaf cuvette at a relative humidity of ca. 50%, a cuvette air flow rate of 300 µmol s^{-1} and an external CO_2 concentration of 360 µl l^{-1}. Irradiance during the measurements was provided by a LED light source (6400-02B, Fa. LI-COR Biosciences) with 10% blue and 90% red light. Light-dependent CO_2 exchange rates (light saturation curves) were surveyed by changing PPFD from 1,500 up to 20 µmol photons m^{-2} s^{-1}. The time to reach the steady state of gas exchange rates for each PPFD was between 2 and 4 min. At the end of each light saturation curve, the dark respiration rates (R_D) were determined after 5 min dark adaptation of the leaf.

The calculation of the gas exchange parameters is based on the equations described by von Caemmerer and Farquhar (1981). According to Webb et al. (1974), the light saturation curves of CO_2 uptake rates were interpolated by a non-linear exponential function (1). The specific constants *A*, *B*, and *C* were estimated by using Newton's least squares method. From these constants, the specific light curve parameters were calculated with Eqs. 2–4: the maximum CO_2 uptake rate (J_{CO_2max}) (2), the dark respiration rate (R_D) (3), and the maximum apparent quantum yield (Φ_{CO_2app}) (4). Φ_{CO_2app} corresponds to the quotient of CO_2 molecules versus the mol incident photons on the leaf surface in the linear region of the light saturation curve.

$$J_{CO_2} = f(\mathrm{PPFD}) = A - \mathrm{e}^{B(-\mathrm{PPFD})} \cdot C, \quad (\mu\mathrm{mol\ CO_2\ m^{-2}\ s^{-1}}) \tag{1}$$

$$J_{CO_2\,max} = A \quad (\mu\mathrm{mol\ CO_2\ m^{-2}\ s^{-1}}), \tag{2}$$

$$R_D = A - C \quad (\mu\mathrm{mol\ CO_2\ m^{-2}\ s^{-1}}), \tag{3}$$

$$\Phi_{CO_2app} = \mathrm{e}^{\ln(A/C)} \cdot B \cdot C \quad [\mathrm{mol\ CO_2 (mol\ photons)^{-1}}]. \tag{4}$$

The CO_2 gas exchange parameters were detected on leaves of intact rice and wheat plants directly after anaerobic incubation for 4, 17, and 24 h, and after 24 h of re-aeration. Additionally, aerated control plants were measured at each time point.

Chlorophyll fluorescence

The chlorophyll (Chl) fluorescence measurements were carried out on attached primary leaves under the respective growth conditions using a portable Chl fluorometer (PAM 2000, Walz, Effeltrich, Germany). For determination of F_o (minimum Chl fluorescence in the dark-adapted state) and F_m (maximum Chl fluorescence in the dark-adapted state), the leaves were dark-adapted for 30 min. The F_v/F_m ratio (=Φ_{max}) was calculated by $(F_m - F_o)/F_m$ after Rohacek (2002). The electron transport rate (ETR) was determined from Chl fluorescence parameters detected on light-adapted leaves as described in Pörs et al. (2001).

These experiments were performed on leaves of intact rice and wheat plants. In one set of assays, measurements were made 30 min after anaerobic incubation (due to the dark adaptation), and after 24 h of anaerobiosis as described for the CO_2 gas exchange parameters.

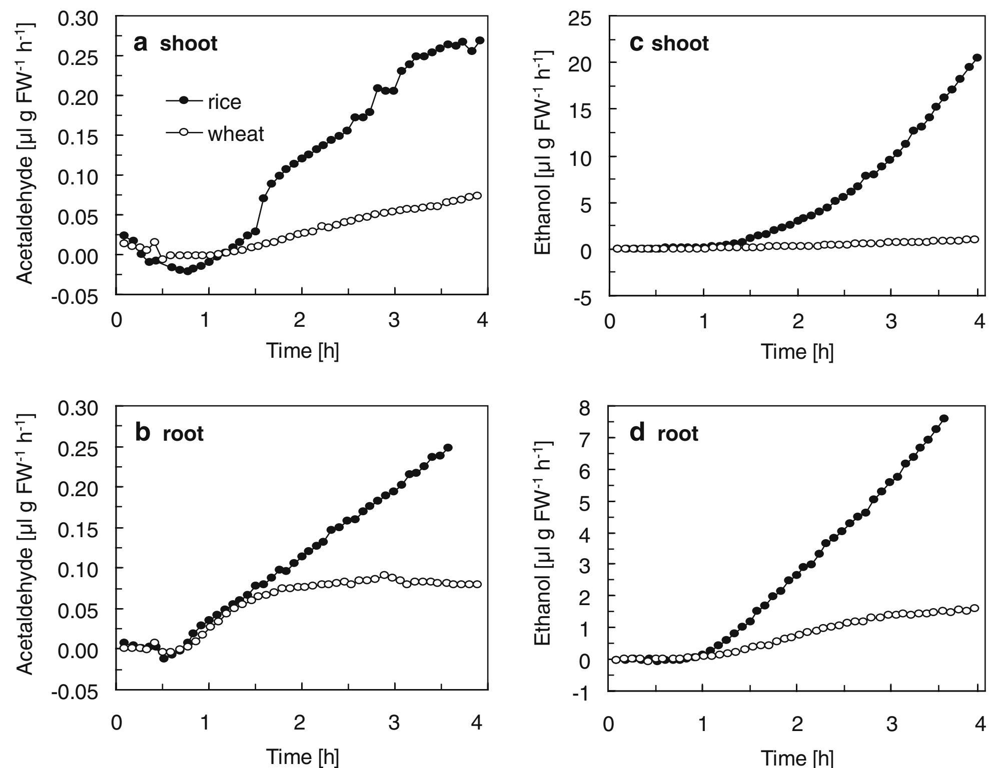

Fig. 1 Roots and shoots fermentation during anaerobic treatment. On-line monitoring of acetaldehyde (**a**, **b**) and ethanol (**c**, **d**) emissions from shoots (**a**, **c**) and roots (**b**, **d**) of intact rice (*closed circles*) and wheat (*open circles*) seedlings exposed to 4 h anaerobic conditions in the light. A two-compartment cuvette (shoots compartment –90 ml and roots compartment –45 ml) was used to monitor simultaneously the root and shoot gas emission. One representative measurement selected from four independent experiments is shown. The height of variation between the measurements is shown in Table 3

In another set of assays, plants were analyzed directly during anaerobiosis. Thereby, Chl fluorescence was measured on plants, which were completely covered with a plastic bag that was fumigated with nitrogen gas or air. This experimental setup allowed application of a buffered solution of 35 mM $KHCO_3$ and 65 mM $NaHCO_3$ (pH 7.5) to the nutrient solution as a CO_2 source.

Statistical analysis

Student's *t*-test ($P<0.05$) upon accomplished *F*-test was used to compare the acetaldehyde, ethanol, and CO_2 production rates, PDC and ADH activities, and metabolites concentrations in rice and wheat roots and shoots, and to determine significance of differences among the calculated values.

Results

Fermentation during a 4-h period of illuminated anaerobiosis

Acetaldehyde and ethanol release was analyzed separately in shoots and roots of intact seedlings, which were exposed to anaerobiosis for 4 h in light (Fig. 1). During this incubation, the partial pressure of oxygen declined below the detection limit of the sensor within 15 min indicating that the plants do not release oxygen upon light exposure (data not shown). After 4 h of anaerobiosis, the CO_2 production rates from shoots were five times lower in wheat (22 µl g FW^{-1} h^{-1}) compared to rice (100 µl g FW^{-1} h^{-1}; Table 1). Thereby, rice and wheat shoots released 65 and 80% less CO_2 during illuminated anaerobiosis than during anaerobic dark incubation (Table 2). In roots, no significant differences were found between light and dark CO_2 emission (Table 2).

Table 1 CO_2 release and uptake rates (µl g FW^{-1} h^{-1}) in aerobic conditions (control), 2 and 4 h after the onset of anaerobic conditions in the light for shoots and roots of rice and wheat seedlings. All values are means of 3–5 individual experiments with standard errors. Values with the same superscript letter do not differ significantly at $P<0.05$ (calculated by the Student's *t*-test)

	CO_2 (µl g FW^{-1} h^{-1})		
	Control	2 h Anaerobiosis	4 h Anaerobiosis
Rice			
Shoot	–1,269± 272[c]	79 ± 41[d]	100 ± 60[d]
Root	182 ± 35[a]	264 ± 43[a]	255 ± 48[a]
Wheat			
Shoot	–568 ± 97[b]	24 ± 10[d]	22 ± 12[d]
Root	275 ± 52[a]	178 ± 23[a]	137 ± 23[a]

Table 2 Ratios of CO_2, acetaldehyde (*AA*) and ethanol (*EtOH*) emission rates, enzyme activities and metabolite contents between anaerobic incubation in light and darkness

	Rice shoot		Rice root		Wheat shoot		Wheat root	
	4 h A	24 h A	4 h A	24 h A	4 h A	24 h A	4 h A	24 h A
CO_2 Emission	0.35		0.9		0.20		0.9	
AA Emission	0.12		1.5		0.07		0.7	
EtOH Emission	0.27		1.7		0.36		0.6	
ADH	1.5	3.0	1.0	0.8	4.3	19.2	1.5	1.9
PDC	1.5	1.5	1.5	0.6	2.1	10.4	1.4	2.4
Glucose	1.1	0.5	1.0	1.5	0.7	0.4	1.0	1.3
Fructose	0.9	0.8	1.3	2.2	0.7	0.2	1.1	1.3
Sucrose	1.5	2.8	1.6	2.5	1.6	4.1	1.5	1.4
Starch	0.9	1.1	0.9	1.9	1.2	1.2	1.3	1.0
Ethanol	0.2	0.4	0.8	0.9	0.6	1.7	0.9	0.8
ATP	1.2	2.6	1.1	3.8	1.6	8.2	1.2	2.6
ADP	1.4	1.7	1.0	4.2	1.5	13.7	1.2	2.1
AEC	1.0	1.4	1.0	1.0	1.0	0.8	1.0	0.9

Measurements were performed with roots and shoots of rice and wheat seedlings after 4 and 24 h of anaerobiosis (*A*). The table shows ratios between the values measured in illuminated anaerobiosis (Tables 1, 3–6) and the respective values measured in darkness (Tables 1–5 from Mustroph et al. 2006). A ratio of 1.0 means no differences between the different treatments

Table 3 Total amounts (in µl g FW^{-1}) of acetaldehyde and ethanol released by shoots and roots of rice and wheat seedlings during 4 h of anaerobic treatment in light

	Acetaldehyde (µl g FW^{-1})	Ethanol (µl g FW^{-1})
Rice		
Shoot	0.52 ± 0.07^{a}	29.18 ± 6.86^{a}
Root	0.61 ± 0.13^{a}	18.62 ± 4.63^{a}
Wheat		
Shoot	0.14 ± 0.06^{b}	1.66 ± 0.31^{c}
Root	0.25 ± 0.03^{b}	3.22 ± 0.30^{b}

These values were calculated from the four independent measurements mentioned in Fig. 1. Values with the same superscript letter in one column do not significantly differ at $P<0.05$ (calculated by the Student's *t*-test)

About 30 min after exposure of intact plants to anaerobiosis, roots started to release acetaldehyde, while ethanol release started 20 min later (Fig. 1b, d). In contrast, shoot acetaldehyde and ethanol release followed 30 min later than that from roots (Fig. 1a, c). Light-exposed and anaerobically incubated rice and wheat shoots released only 27 and 36% of the amount of ethanol during dark incubation after 4 h (Table 2). Moreover, very little acetaldehyde was emitted from shoots of either species (Table 3). In contrast, root ethanol and acetaldehyde releases of light-incubated seedlings were more similar to those of dark-incubated seedlings after 4 h (Table 2).

Although plants displayed lower fermentation rates during anaerobiosis in light than in darkness, illuminated rice shoots still emitted 3.7 times more acetaldehyde and 18 times more ethanol within a 4 h incubation period than light-exposed wheat shoots (Table 3). Furthermore, rice roots released 2.4 times more acetaldehyde and six times more ethanol than wheat roots during illuminated anaerobiosis (Table 3).

Ethanol production during longer duration of anaerobiosis under different light regimes

In addition to the 4-hour-experiments, the fermentation products were measured, which were emitted from whole seedlings grown anaerobically for a 20-hour-period. Very low levels of acetaldehyde release were detected either from rice or wheat plants during light exposure (Fig. 2a). The ethanol release of illuminated rice seedlings started after 2 h, increased within the next 5 h, and was almost stable during the remaining anaerobic period (Fig. 2b). After 10 h of anaerobiosis, ethanol release of rice plants was seven times higher compared to wheat (10 µl g FW^{-1} h^{-1} vs. 1.4 µl g FW^{-1} h^{-1}).

A light–dark cycle during anaerobic incubation revealed different results. Seedlings were initially illuminated for 10 h and, subsequently, placed in darkness. Transition from light to darkness during anaerobiosis resulted in a sudden increase of acetaldehyde release and subsequently in an additional increase of ethanolic fermentation in both plant species (Fig. 2c, d). The amounts of acetaldehyde (0.26 µl g FW^{-1} h^{-1}) and ethanol (21 µl g FW^{-1} h^{-1}) produced by rice at the end of the dark period were comparable to those produced in continuous darkness (0.28 and 27 µl g FW^{-1} h^{-1}, respectively, from Mustroph et al. 2006). However, wheat released two times more ethanol in the dark

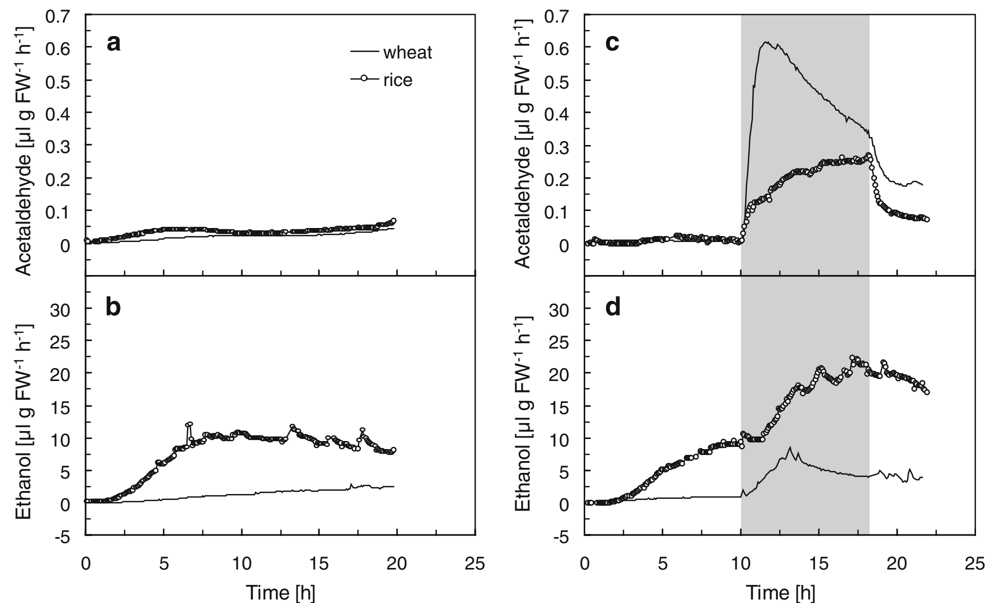

Fig. 2 Long term fermentation in whole plants. Acetaldehyde (**a**, **c**) and ethanol (**b**, **d**) emissions during anaerobiosis in different light regimes were detected by the photoacoustic method from whole wheat (*simple line*) and rice (*line with circles*) plants. Measurements were made under continuous illumination (**a**, **b**) or alternately with illumination and darkness (*gray* in **c**, **d**). One representative measurement selected from three independent experiments is shown

period after an anaerobic incubation in light than after a 20-hour-incubation in continuous darkness (4.5 and 2.0 µl g FW^{-1} h^{-1}, respectively). When seedlings were re-exposed to light, their acetaldehyde emission decreased again, but ethanol release remained longer at a level, which was equivalent to the dark level.

Activities of the fermentative enzymes pyruvate decarboxylase and alcohol dehydrogenase

The in vitro activities of the fermentative enzymes ADH and PDC of rice and wheat roots, based on the total protein content, were higher during normoxic conditions than the shoot activities of both plant species (Table 4). However, rice shoots contained 4–5 times higher fermentative enzyme activities than wheat shoots. During 4 h anaerobiosis, the activities of ADH and PDC already increased by 1.5–3 times in all tissues. When anaerobiosis in a light–dark cycle continued, ADH and PDC activities were continuously stimulated and reached 5–10 times higher levels compared to aerobic conditions in rice and wheat (Table 4). It is worth to mention that rice shoots contained 5 and 10 times higher ADH and PDC activities after a 24 h period of anaerobiosis than wheat, while in roots these enzyme activities did not significantly differ between these species (Table 4).

Nevertheless, in light-exposed wheat shoots, the ADH and PDC activities were four and twofold higher after a 4 h anaerobic period in comparison to dark incubation, and 19- and 10-fold higher after 24 h of anaerobiosis (Table 2). This difference in fermentative enzyme activities between light and darkness was neither observed in wheat roots nor in roots and shoots of rice plants.

Table 4 Activities of the fermentative enzymes ADH and PDC (nmol mg $protein^{-1}$ min^{-1}) in roots and shoots of rice and wheat plants during aerated control (0 h) and after 4 and 24 h of anaerobiosis (*A*) in the light–dark cycle

		ADH (nmol mg $protein^{-1}$ min^{-1})	PDC (nmol mg $protein^{-1}$ min^{-1})
Rice			
Shoot	0 h	115 ± 17^{a}	2.84 ± 0.66^{a}
	4 h A	231 ± 22^{b}	10.83 ± 1.95^{b}
	24 h A	687 ± 102^{c}	24.77 ± 3.64^{c}
Root	0 h	279 ± 67^{b}	9.00 ± 2.29^{b}
	4 h A	930 ± 294^{d}	19.18 ± 2.00^{c}
	24 h A	988 ± 269^{d}	52.81 ± 9.94^{d}
Wheat			
Shoot	0 h	26 ± 7^{e}	0.59 ± 0.28^{e}
	4 h A	44 ± 6^{e}	1.08 ± 0.14^{a}
	24 h A	149 ± 23^{a}	2.50 ± 0.28^{a}
Root	0 h	435 ± 99^{b}	6.43 ± 1.82^{b}
	4 h A	622 ± $212^{b,c}$	13.39 ± 2.22^{b}
	24 h A	1,012 ± 195^{d}	35.53 ± 4.69^{d}

All values are means of 6–10 samples with standard errors. Values with the same superscript letter in one column do not significantly differ at $P < 0.05$ (calculated by the Student's *t*-test)

Carbohydrate depletion and ethanol accumulation inside the tissue

The levels of carbohydrates consumed during anaerobiosis were analyzed in shoots and roots of both plant species. During aerobic conditions, rice shoots contained three times more sucrose than wheat (Table 5). About 4 h light exposure of seedlings during anaero-

Table 5 Contents of soluble carbohydrates, starch (mg g FW^{-1}) and ethanol (μg g FW^{-1}) in roots and shoots during aerated control (0 h) and after 4 and 24 h of anaerobiosis (*A*) in the light–dark cycle

		Glucose (mg g FW^{-1})	Fructose (mg g FW^{-1})	Sucrose (mg g FW^{-1})	Starch (mg g FW^{-1})	Ethanol (μg g FW^{-1})
Rice						
Shoot	0 h	0.49 ± 0.04^{a}	0.51 ± 0.04^{a}	7.27 ± 0.32^{a}	0.51 ± 0.04^{a}	10 ± 1^{a}
	4 h A	0.26 ± 0.02^{b}	0.27 ± 0.02^{b}	3.74 ± 0.16^{b}	0.28 ± 0.03^{b}	42 ± 8^{b}
	24 h A	0.23 ± 0.03^{b}	0.19 ± 0.03^{b}	1.56 ± 0.15^{d}	0.19 ± 0.02^{c}	118 ± 17^{c}
Root	0 h	0.06 ± 0.02^{c}	0.17 ± 0.04^{c}	0.93 ± 0.09^{d}	0.14 ± 0.01^{c}	4 ± 2^{d}
	4 h A	0.03 ± 0.01^{c}	0.15 ± 0.04^{c}	0.43 ± 0.14^{e}	0.11 ± 0.03^{c}	4 ± 2^{d}
	24 h A	0.03 ± 0.01^{c}	0.06 ± 0.01^{c}	$0.22 \pm 0.04^{e,f}$	0.14 ± 0.01^{c}	4 ± 1^{d}
Wheat						
Shoot	0 h	0.59 ± 0.08^{a}	0.30 ± 0.03^{b}	2.67 ± 0.16^{c}	0.71 ± 0.07^{d}	16 ± 2^{a}
	4 h A	0.36 ± 0.05^{b}	0.18 ± 0.02^{c}	0.95 ± 0.07^{d}	0.27 ± 0.03^{b}	41 ± 8^{b}
	24 h A	$0.45 \pm 0.07^{a,b}$	0.10 ± 0.02^{c}	0.80 ± 0.13^{d}	0.31 ± 0.05^{b}	84 ± 5^{c}
Root	0 h	0.18 ± 0.02^{b}	0.24 ± 0.04^{b}	0.74 ± 0.07^{d}	0.10 ± 0.01^{c}	14 ± 2^{a}
	4 h A	0.10 ± 0.02^{d}	0.12 ± 0.02^{c}	0.29 ± 0.08^{e}	0.09 ± 0.02^{c}	12 ± 2^{a}
	24 h A	0.03 ± 0.01^{c}	0.03 ± 0.01^{d}	0.15 ± 0.02^{f}	0.08 ± 0.02^{c}	12 ± 2^{a}

All values are means of 5–10 samples with SE. Values with the same superscript letter in one column do not significantly differ at $P < 0.05$ (calculated by the Student's *t*-test)

biosis resulted in 50 and 60% decrease in sucrose content in rice and wheat plants, respectively. During the following 20 h of anaerobiosis, sucrose contents further declined leading to final concentrations of 20% (rice) and 35% (wheat) of aerated control levels. Starch degradation was only observed in shoot tissue during the anaerobic period (Table 5).

Despite the lower fermentation rates of seedlings during light-exposed anaerobiosis, carbohydrate contents decreased to almost the same extent than in complete darkness (Table 2). Thereby, shoots of the seedlings contained less glucose and fructose, but higher sucrose amounts after 24 h anaerobic incubation in light than in darkness.

In addition to the measurement of ethanol release from plant tissues, the ethanol content was measured within the plant tissues to examine differences in excretion rates between the species and the different tissues. Roots did not accumulate ethanol inside the tissue during anaerobiosis (Table 5). Shoots, however, accumulated ethanol during the 24 h-period of illuminated anaerobiosis, reaching 11 and five times higher amounts than in aerated rice and wheat shoots, respectively (Table 5). Comparing the species, rice shoots contained 40% more ethanol than wheat shoots after 24 h without oxygen.

Survival rates and ATP content after anaerobic treatment

Wheat plants did not survive a period of 24 h of anaerobiosis in darkness, and rice plants exhibited severe cellular damage (Mustroph et al. 2006). The survival rate of both species to oxygen deficiency was enhanced by light exposure, although wheat was again more sensitive to anaerobiosis than rice. During a 24 h treatment of illuminated anaerobiosis, rice did not show any leaf damage, but wheat shoots showed necroses in the upper part of the leaves (Fig. 3b).

The ATP contents in shoots of rice and wheat decreased by around 20% during the first 4 h of illuminated anaerobiosis in comparison to the aerated control condition (Table 6). After 24 h of anaerobiosis, the ATP levels in wheat shoots declined to 56% and remained almost stable in rice shoots with 90% of the aerated control levels. In roots of rice and wheat, the ATP content already decreased to 71 and 56% of aerated control levels, respectively, after 4 h of illuminated anaerobiosis (Table 6). After 24 h, the roots contained only 36 and 16% ATP in comparison to the aerated roots. The AEC remained in a physiological range in all tissues during the observed time period, indicating a high regulatory capacity of the adenylate pool (Table 6).

Compared to the anaerobic incubation in darkness, rice shoots contained 2.6 times more ATP after 24 h of illuminated anaerobiosis, but wheat shoots possessed 8.2 times more ATP (Table 2). In rice and wheat roots, a 3.8- and 2.6-fold higher ATP content was determined after 24 h anaerobiosis in light than in darkness.

Pigment contents in leaves after anaerobic treatment

Contents of Chl and xanthophylls were measured in rice and wheat leaves after 24 h anaerobiosis in a light–

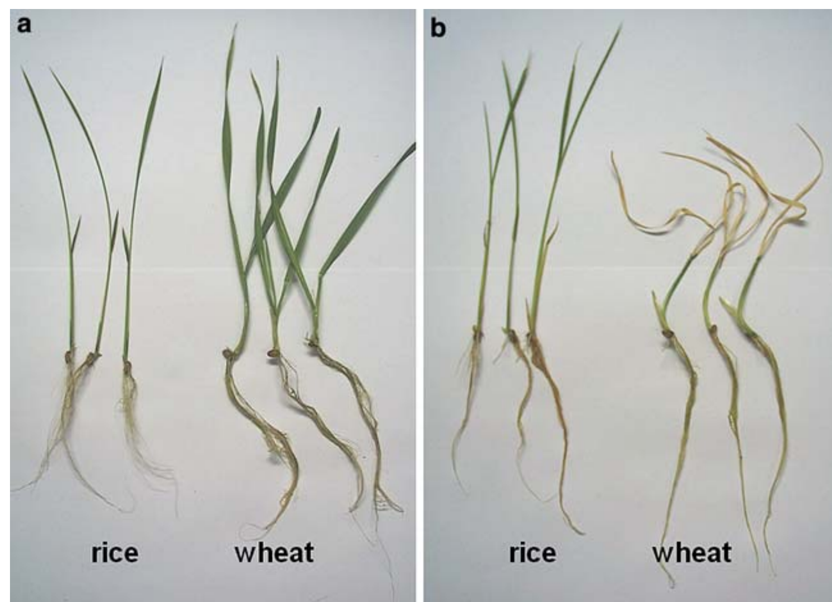

Fig. 3 Fourteen-day-old rice and nine-day-old wheat seedlings after 24 h anaerobic treatment in the light–dark cycle, followed by growth in aerated conditions for further 3 days (**b**), and continuously aerated plants (**a**)

Table 6 Contents of ATP and ADP (nmol g FW^{-1}) and AEC in roots and shoots during aerated control (0 h) and after 4 and 24 h of anaerobiosis (*A*) in the light-dark cycle

		ATP (nmol g FW^{-1})	ADP (nmol g FW^{-1})	AEC (rel U)
Rice				
Shoot	0 h	91.0 ± 5.1[a]	61.9 ± 6.3[a]	0.78 ± 0.02[a]
	4 h A	70.0 ± 6.8[b]	64.4 ± 7.7[a]	0.71 ± 0.01[b]
	24 h A	84.7 ± 4.8[a]	54.5 ± 5.3[a]	0.78 ± 0.02[a]
Root	0 h	39.5 ± 2.1[c]	23.6 ± 2.1[b]	0.79 ± 0.02[a]
	4 h A	31.4 ± 5.5[c]	12.0 ± 1.3[c]	0.83 ± 0.03[a]
	24 h A	14.4 ± 1.4[f]	14.3 ± 2.1[c]	0.66 ± 0.04[b]
Wheat				
Shoot	0 h	52.4 ± 3.7[d]	45.0 ± 2.4[d]	0.66 ± 0.03[b]
	4 h A	44.2 ± 2.5[c]	45.6 ± 4.4[d]	0.66 ± 0.02[b]
	24 h A	30.6 ± 4.4[c]	36.8 ± 2.6[b]	0.55 ± 0.04[c]
Root	0 h	44.2 ± 2.3[c]	21.9 ± 1.9[b]	0.80 ± 0.03[a]
	4 h A	24.8 ± 3.4[e]	13.1 ± 1.1[c]	0.79 ± 0.02[a]
	24 h A	7.3 ± 1.1[g]	5.1 ± 0.8[e]	0.61 ± 0.04[b]

All values are means of 5–10 samples with SE. Values with the same superscript letter in one column do not significantly differ at $P < 0.05$ (calculated by the Student's *t*-test)

dark cycle. The amounts of Chl in rice and wheat leaves were not affected during the anaerobic incubation period (Table 7). The xanthophyll pool size was only slightly changed during anaerobiosis: the amount of all xanthophylls in rice leaves was around 15% higher under anaerobic compared to aerated conditions, but not altered in wheat leaves. However, the ratio of different xanthophylls was drastically changed. While the amount of violaxanthin was dramatically reduced in rice and wheat under anaerobiosis, the zeaxanthin content increased 17 times (Table 7). Therefore, the DEPS increased in both plant species about 15 times during anaerobic incubation compared to the aerated conditions.

CO_2 gas exchange and Chl fluorescence after anaerobic incubation

To assess any damage of the photosynthetic apparatus through low oxygen stress, we analyzed several

Table 7 Pigment contents (mg g FW^{-1}), Chl a/b ratio, and DEPS in leaves of rice and wheat plants grown in aerated control (*C*) compared to treatment with anaerobiosis (*A*) for 1 day in the light–dark cycle

	Chl a (mg g FW^{-1})	Chl b (mg g FW^{-1})	Chl a/b (rel U)	Violaxanthin	Antheraxanthin (mg g FW^{-1})	Zeaxanthin	DEPS (rel U)
Rice							
C	924 ± 136[a]	325 ± 52[a]	2.88 ± 0.07[a]	55.9 ± 9.7[a]	3.4 ± 0.1[a]	2.0 ± 0.3[a]	0.07 ± 0.01[a]
1 day A	942 ± 76[a]	327 ± 23[a]	2.88 ± 0.09[a]	5.1 ± 1.5[b]	2.6 ± 1.3[a]	59.2 ± 5.4[b]	0.90 ± 0.03[b]
Wheat							
C	1,028 ± 39[a]	380 ± 21[a]	2.72 ± 0.11[a]	51.6 ± 2.9[a]	3.6 ± 0.7[a]	1.5 ± 0.8[a]	0.05 ± 0.02[a]
1 days A	878 ± 64[a]	333 ± 43[a]	2.72 ± 0.15[a]	9.2 ± 0.7[b]	3.9 ± 1.6[a]	39.9 ± 5.9[c]	0.79 ± 0.01[c]

Values are means of six samples with SE. Values with the same superscript letter in one column do not significantly differ at $P < 0.05$ (calculated by the Student's *t*-test)

photosynthetic parameters 30 min and 24 h after different times of anaerobic incubation in the following re-aeration period. Within the first 5 h anaerobiosis in light, the maximum net CO_2 uptake rate ($J_{CO_{2max}}$) under aeration declined in rice and wheat, whereas the loss was more dramatic in rice compared to wheat (74 vs. 25% of the control values, Fig. 4a). But the 5 h level of $J_{CO_{2max}}$ of rice seedlings remained stable during the next 20 h anaerobiosis in day–night cycle, while $J_{CO_{2max}}$ of wheat dropped below zero (Fig. 4a).

The apparent quantum yield of CO_2 uptake ($\Phi_{CO_{2app}}$) indicates the maximum use of photons for CO_2 uptake. This value was also more stable in rice than in wheat after 24 h of anaerobiosis (Fig. 4b). After re-aeration for 24 h, rice seedlings recovered from anaerobiosis and increased $J_{CO_{2max}}$ and $\Phi_{CO_{2app}}$, while wheat leaves did not improve both measured photosynthesis activities (Fig. 4a, b). The dark respiration rates were not significantly changed after different periods of anaerobic treatment (Fig. 4c).

For the evaluation of functioning and integrity of PSII reaction centers after low oxygen stress, we determined Chl fluorescence 30 min and 24 h after anaerobiosis. The maximum quantum yield of PSII photochemistry, an indicator for possible PSII impairments, was decreased to 40 and 15% of aerated levels

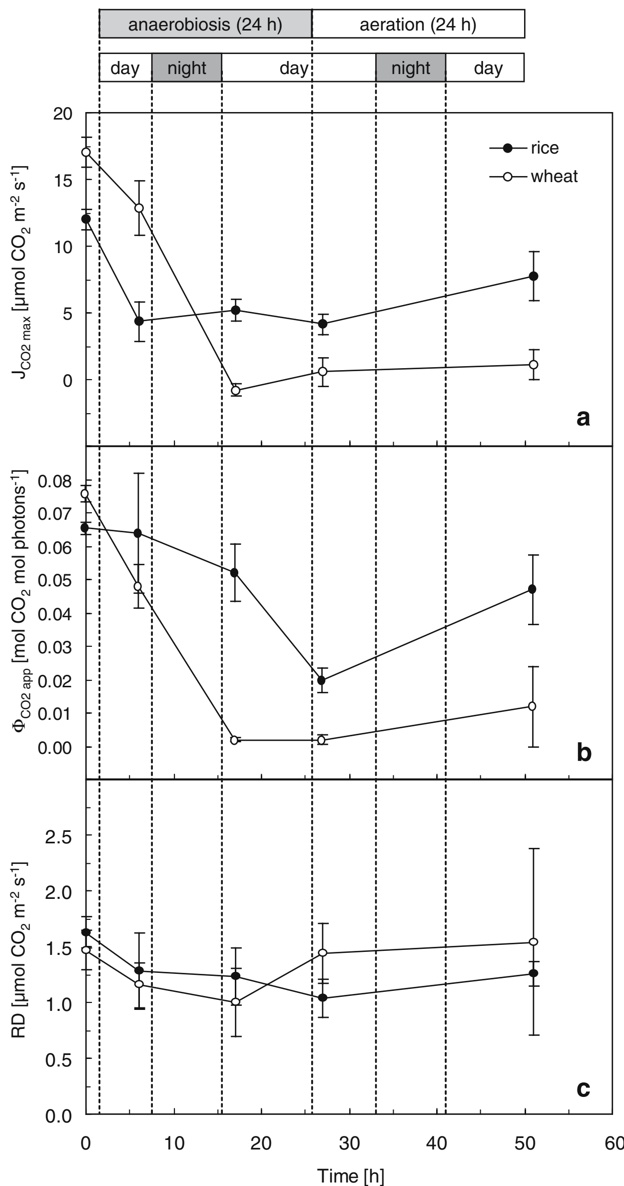

Fig. 4 Photosynthesis in leaves after anaerobiosis. Rice and wheat seedlings were incubated in anaerobiosis for 4, 17, and 24 h in a light–dark cycle. Thirty minutes after anaerobiosis, maximum CO_2 gas exchange (**a**), maximum apparent quantum yield of CO_2 uptake (**b**), and dark mitochondrial respiration (R_D, **c**) were measured in air. Additionally, these values were detected after subsequent re-aeration for 24 h. Values are the means with SE of 4–8 samples from three independent experiments

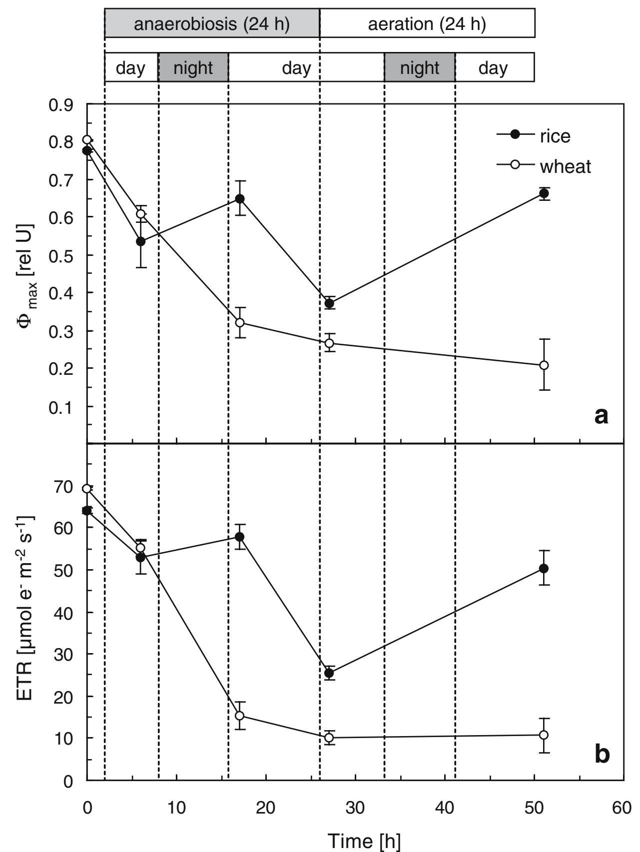

Fig. 5 Chlorophyll fluorescence in leaves after anaerobiosis. Rice and wheat seedlings were incubated in anaerobiosis for 4, 17, and 24 h in a light–dark cycle. Thirty minutes after anaerobiosis, maximum quantum yield of PSII photochemistry (Φ_{max}, **a**) and electron transport rate (*ETR*, **b**) were measured in air. Additionally, these values were detected after subsequent re-aeration for 24 h. Values are the means with SE of 10–20 samples from three independent experiments

in rice and wheat after a 24 h period of anaerobiosis, respectively (Fig. 5a). The ETR under current growth conditions followed similar kinetics in wheat and rice during anaerobiosis in the light–dark cycle (Fig. 5b). Φ_{max} and ETR recovered within 24 h re-aeration in rice seedlings, but not in wheat seedlings.

CO_2 gas exchange and Chl fluorescence during anaerobic incubation

A main limiting factor for continuing photosynthesis in leaves during anaerobiosis is CO_2 uptake of seedlings. Due to our experimental setup, the anaerobic atmosphere contained only nitrogen gas without additional supply of CO_2. Therefore, no net CO_2 uptake was measurable in the nitrogen flow (Table 1). However, internal cycles of CO_2 and O_2 generation and consumption in photosynthetic and respiratory processes inside the leaves cannot be excluded. Therefore, the intactness of the PSII reaction centers and the rate of photosynthetic electron transport were tested by measuring Chl fluorescence during anaerobiosis.

The Chl fluorescence analysis in the nitrogen atmosphere in light revealed that Φ_{max} was hardly affected during short-term anaerobiosis, and decreased to 85% of control values after 6 h anaerobiosis (Fig. 6a, b). But the ETR declined already within 30 min of anaerobiosis to less than one third of the control value determined in ambient air conditions in both species (Fig. 6c, d). Interestingly, wheat seedlings showed less impairment with a 65% reduction of ETR than rice seedlings with a 75% reduction of ETR (Fig. 6c, d).

Because of the CO_2 limitation of anaerobic photosynthesis, we also analyzed Chl fluorescence of rice and wheat seedlings in nitrogen atmosphere in combination with a nutrient solution supplemented with a CO_2 source, a mixture of $NaHCO_3/KHCO_3$. Under control with ambient air, no differences were found with or without additional CO_2 supply (data not shown). After 4 h anaerobiosis with additional CO_2 supply, the ETR was reduced only by 30 and 20% of the aerated control values of rice and wheat, respectively (Fig. 6c, d). Furthermore, Φ_{max} did not significantly decrease in both anaerobically incubated plant species upon the additional CO_2 source (Fig. 6a, b).

Discussion

Light lowers the need for fermentation activity in the shoots and enhances survival

The aim of these studies was to examine in detail the ATP generating processes of tolerant and sensitive

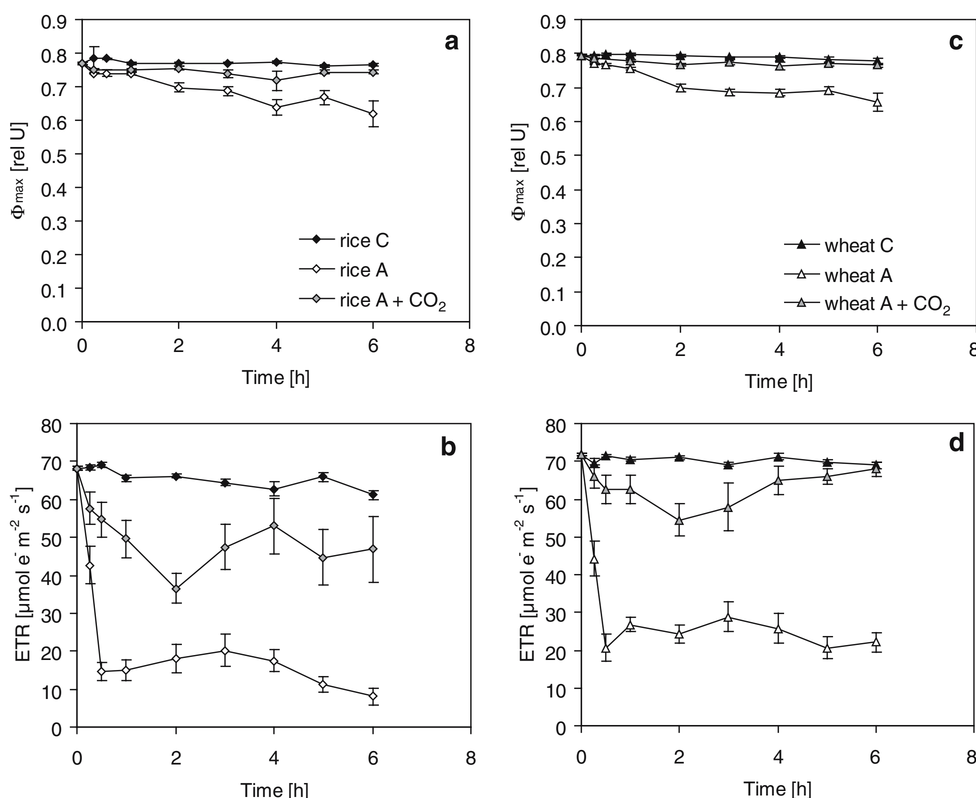

Fig. 6 Chlorophyll fluorescence in leaves during anaerobiosis. Maximum quantum yield of PSII photochemistry Φ_{max}, **a**, **c**) and electron transport rate (*ETR*, **b**, **d**) were measured directly on anaerobically treated leaves of rice (**a**, **b**) and wheat plants (**c**, **d**). *C* aerated control; *A* anaerobiosis; *A* + CO_2 A mixture of $KHCO_3$ and $NaHCO_3$ was given to the nutrient solution as a CO_2 source. Values are the means with SE of 10–20 samples from three independent experiments

plant species under anaerobiosis during light exposure in different organs. From earlier experiments, it was supposed that light favors the survival of plants in anaerobiosis. Ram et al. (2002) demonstrated that turbid floodwater decreases the survival rate of rice plants in comparison to clear floodwater. Similarly, light increased the tolerance of rice seedlings to nitrogen atmosphere from 24 h to about 4 days (Mustroph and Albrecht 2003). Our experiments confirmed that illumination enhances the survival rate of rice and wheat plants during oxygen deprivation (Fig. 3 versus Fig. 4 in Mustroph et al. 2006). Accordingly, the ATP levels in shoots of both species were 2.6 and 8.2 times higher after 24 h anaerobiosis in light than in darkness (Table 2).

Analysis of light-exposed plants revealed that ethanol emission of rice and wheat shoots was lowered by 73 and 64% during 4 h anaerobiosis, respectively, in comparison to darkness (Table 2), and confirmed and specified previous results for whole rice plants (Boamfa et al. 2003). Furthermore, rice and wheat shoots released 65 and 80% less CO_2 in the light compared to dark anaerobic incubation (Table 2). These observations account for a reduced ethanol fermentation rate in shoots during light-exposed anaerobiosis, although low oxygen stress increased in vitro fermentative enzyme activities to a similar extent (rice) or to even elevated levels (wheat) than under anaerobiosis in darkness (Tables 2, 4). Furthermore, the low CO_2 release could point to a recycling of respiratorily produced CO_2 in the photosynthetic process. These data suggest that apart from glycolysis, other ATP-generating processes, mainly parts of the photosynthesis, occur during anaerobiosis in the presence of light. Thus, although fermentation is partially reduced in shoots, the plants can survive better under these conditions.

Wheat shoot fermentation activity in darkness benefits from previous illumination

Transfer of wheat seedlings during anaerobiosis after 10 h light incubation to darkness resulted in a twofold higher ethanol production than during continuous dark anaerobiosis (Fig. 2 versus Fig. 3 from Mustroph et al. 2006). This observation is supported by a twofold induction of fermentative enzyme activities in wheat shoots after 4 h of illuminated anaerobiosis in comparison to aeration, and a four- to fivefold induction after 24 h anaerobiosis in the light–dark cycle (Table 4), while these activities decreased in darkness (Table 3 from Mustroph et al. 2006). We conclude that light during the anaerobic incubation stimulates fermentation capacity of wheat seedlings in a subsequent dark period.

Enhanced induction of fermentative enzymes in light could have different reasons. First, light could be essential for the induction of genes encoding fermentative enzymes during anaerobiosis in leaves. In fact, the need of light for anaerobically induced gene expression was previously presented for the maize GapC4 promoter, which was strongly induced upon anaerobiosis only under light exposure (Hänsch et al. 2003). Second, the considerably elevated ATP level in wheat shoots under illuminated anaerobiosis in comparison to darkness (Table 2) provides more energy resources to promote expression of essential proteins.

Rice is more tolerant to anaerobiosis than wheat during illumination

Under illuminated anaerobiosis, ethanolic fermentation of rice seedlings was at least 73% lower in comparison to darkness (Table 2). However, even during illumination, rice shoots released 18 times more ethanol than wheat shoots (Table 3). A similar difference of ethanolic fermentation rate was observed between rice and wheat seedlings during dark anaerobiosis (Mustroph et al. 2006). This elevated fermentation capacity enables improved energy supply and, thus, better survival during oxygen deficiency in rice plants compared to wheat (Fig. 3, Table 6). Accordingly, rice shoots contained almost the same ATP content and an equal AEC value after 24 h of illuminated anaerobiosis as under aerobic conditions. In contrast, wheat shoots showed a 42% decrease in the ATP content and 17% decrease of AEC (Table 6). The high fermentation rate of rice shoots, which became obvious by comparison of the ethanol production rates (Figs. 1, 2, Tables 3, 5) as well as of the CO_2 release (Table 1), is most likely due to a twofold higher sugar content of rice than of wheat shoots in ambient air conditions (Table 5) and four- to fivefold higher ADH and PDC activities in rice than in wheat shoots during anaerobic incubation (Table 4).

The improved tolerance against anaerobiosis of light-exposed rice seedlings in comparison to wheat became additionally evident by the analysis of photosynthetic parameters. After a 24 h period of oxygen deprivation, the capacity for photosynthesis decreased less drastically in rice seedlings than in wheat (Fig. 4). Moreover, the photosynthetic capacity also recovered faster and completely during re-aeration in rice seedlings. All calculated photosynthetic parameters confirmed the better fitness of rice shoots compared to wheat (Figs. 4, 5).

Nevertheless, 24 h of anaerobiosis resulted in a lower photosynthetic capacity in the re-aeration period in both species. The quantum yield of CO_2 fixation as well as the ETR decreased in rice shoots by 70 and 65%, and in wheat shoots by 95 and 85%, respectively (Figs. 4b, 5b). These findings are consistent with a general down-regulation of the primary metabolism in response to energy deficiency, a lack of CO_2 for photosynthesis due to stomata closure after root injuries (Jackson et al. 2003), and oxidative damages of the photosynthetic apparatus due to re-aeration (Rawyler et al. 2002; Schlüter and Crawford 2003). The increased DEPS of wheat and rice shoots after 24 h of anaerobiosis indicate photooxidative stress in comparison to the aerated control conditions (Table 7). However, the higher DEPS and the higher amounts of zeaxanthin in rice than in wheat are indicative of an improved potential of non-photochemical dissipation of the excessive excitation energy.

Inhibition of photosynthesis after anaerobic incubation in darkness has been reported for *Acorus calamus*, *Iris pseudacorus*, and *Vaccinium macrocarpon* (Schlüter and Crawford 2001, 2003). As Chl content was not reduced during anaerobiosis (Table 7), limitation of Chl cannot be attributed to reduced photosynthesis capacity.

Not only the shoots, but also the rice roots apparently benefit from light exposure, as the roots contain 3.8 times more ATP than after dark anaerobiosis (Table 2). However, while light slowed down ethanol production in shoots, the roots of rice seedlings excreted two times more ethanol than during darkness (Tables 2, 3). The additional capacity of ethanol production could presumably be assigned to a higher sugar transport rate from shoots to roots during illumination. The higher ATP content in illuminated tissues compared to darkness (Tables 2, 4) could enable this process, as the phloem transport is normally inhibited in low oxygen stress due to energy deficiency (Dongen et al. 2003).

In contrast to rice, wheat roots did not benefit much from light treatment during anaerobiosis (Table 6). Comparison of biomass production of shoots and roots of *Rumex* species during illuminated and dark submergence also confirmed that these roots did not benefit from illumination as much as shoots (Nabben et al. 1999).

ATP generation during anaerobiosis

Illumination of both species in anaerobiosis resulted in an about 70% lower ethanol release rate in shoots of both species (Table 3) and less ethanol storage inside the shoot tissue (Table 5) compared to dark anaerobiosis (Table 2). But the depletion of sugars was only 10% lower in rice and 20% higher in wheat during illumination compared to darkness after 4 h of anaerobiosis (Table 2). We assume additional sugar-consuming and ATP-generating pathways. Shoots of rice and wheat plants kept the ATP levels 2.6- and 8.2-fold higher during a 24 h period of anaerobiosis in illumination than in darkness (Table 2). It is possible that among the ATP-generating pathways, mitochondrial respiration partly contributed to the ATP formation by consuming photosynthetically produced oxygen. The ability for mitochondrial respiration was not inhibited in both plant species during 24 h anaerobiosis as the value for R_D shows (Fig. 4c), in contrast to the impairment of photosynthetic parameters (Figs. 4a, b, 5).

During the anaerobic incubation in pure nitrogen, both plants neither released additional oxygen upon light exposure nor took up CO_2 (Table 1). Furthermore, the ETR around PSII was strongly inhibited (Fig. 6). These data suggest a low photosynthetic activity for anaerobically stressed plants. Our results are in agreement with those of Ivanov and Edwards (2000), who showed a reduced PSII yield, which is a value for electron transport through PSII, in anaerobic maize chloroplasts confirming that photochemical processes are diminished during anaerobiosis.

The lower ETR during anaerobiosis might be due to over reduction of the photosynthetic electron transfer chain. This was most likely caused by CO_2 limitation. The block of the electron transport chain resulted in a massive over reduction of PSII during anaerobic exposure in light. It was shown for several plant species that low oxygen concentrations resulted in high Chl fluorescence as an indication for a highly reduced plastochinone pool (*Phaseolus vulgaris* L., *Pisum sativum* L., and *Helianthus annuus* L., Farineau 1999). The activated de-epoxidation of the xanthophyll cycle (Table 7) is most likely a consequence of over excitation of PSII.

Apart from low oxygen concentration, a main reason for the tailback of electrons in the ETR is CO_2 deficiency. The anaerobic ETR was stimulated by additional CO_2 supply (Fig. 6c). Positive effects of bicarbonate were previously shown for thylakoid photosynthesis (Sundby 1990) and *Chlamydomonas* cells (Antal et al. 2004) under anaerobiosis. Additionally, bicarbonate enhances submergence photosynthesis in intact *Potamogeton* leaves (Frostchristensen and Sandjensen 1995) and favors survival of submerged rice plants (Krishnan and Ramakrishnayya 1999) as well as of barley plants during anaerobiosis (van der Heide et al. 1963).

However, CO_2 deficiency was apparently not a single factor limiting the ETR, since photosynthetic electron transport was still lower upon CO_2 feeding in anaerobiosis compared to aeration (Fig. 6). This CO_2-independent decrease of ETR may coincide with NADH accumulation during glycolysis and ATP deficiency caused by anaerobiosis (Table 6).

As a consequence of a lower ETR (Fig. 6), the oxygen generation is also most likely impaired during anaerobiosis in illuminated rice and wheat shoots. When pyridine nucleotides, adenylates, and CO_2 limit photosynthesis during anaerobiosis, an alternative electron transport is proposed for ATP production in addition to glycolytic and photo-phosphorylation: when PSII activity is strongly inhibited during anaerobiosis, a cyclic electron transport around PSI could contribute to ATP production (Antal et al. 2003). Besides the common cyclic electron transport via the ferredoxin-PQ reductase, an alternative route was suggested (Ravenel et al. 1994) including an electron flow from NADPH to the PQ pool via a transhydrogenase and the NAD(P)H-PQ-oxidoreductase complex (NDH). Current models of chlororespiration are reviewed in detail by Peltier and Cournac (2002).

This latter cycle is supposed to be active during anaerobiosis in *Chlamydomonas* cells (Antal et al. 2003; Posewitz et al. 2004). Similar mechanisms presumably also exist in higher plants. Cyclic electron transport activity during anaerobiosis was shown for several C_3 plant species (Haldimann and Strasser 1999; Joet et al. 2002; Garstka et al. 2004). Joet et al. (1998) confirmed that NDH is involved in this cycle. They demonstrated that tobacco mutants lacking the NDH protein complex do not show cyclic electron transport during anaerobiosis. Therefore, we assume that the NDH-dependent pathway of cyclic electron transport produces energy during illuminated anaerobiosis in rice and wheat plants. This mechanism would supply ATP for anaerobically treated plants leading to maintenance of the AEC in a physiological range (Table 6). In agreement to the model, it is reasonable to assume that NADH produced by dissimilatory processes directs electrons also into the plastidic PQ pool. This hypothesis is further supported by the fact that the sugar depletion rate during anaerobiosis was nearly as high during light as during dark incubation, unless ethanol production was lower (Table 2).

Future investigations should confirm the activity of this cyclic electron transport in chloroplasts during anaerobiosis in rice and wheat seedlings. Furthermore, it is at present unknown to which extent the Calvin cycle is still working during anaerobiosis using the supplemented CO_2 or CO_2 from mitochondrial respiration.

Conclusions

These experiments provide evidence that illumination during anaerobiosis extends the survival of plants. Ethanolic fermentation rate is lower during light exposure of plants than in darkness indicating that other energy-generating processes can substitute respiration during anaerobiosis for long-term ATP requirement. The photosynthesis functions only to a low extent during oxygen deficiency, which is mainly due to CO_2 deficiency. It is proposed that cyclic electron transport could at least partially contribute to ATP production, when lack of CO_2 and inhibited mitochondrial respiration occur during anaerobiosis. These metabolic interactions of the primary metabolism need further examinations. In addition, the measurements should be also performed under anaerobiosis at different CO_2 concentrations to analyze more natural flooding conditions.

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