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Diminution of photosynthesis in rice (*Oryza sativa* L.) seedlings under elevated CO_2 concentration and increased temperature

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Abstract

The photosynthetic responses to elevated CO_2 concentration (EC) at ambient and ambient +4°C temperature were assessed in the second leaf of rice (*Oryza sativa* L.) seedlings. The duration of different leaf developmental phases, as characterised by changes in photosynthetic pigment contents and photochemical potential, was protracted in the seedlings grown under EC. On the other hand, a temporal shift in the phases of development with an early onset of senescence was observed in the seedlings grown under EC at ambient +4°C temperature. The contents of carotenoids, β-carotene, and xanthophyll cycle pigments revealed that EC downregulated the protective mechanism of photosynthetic apparatus against oxidative damages, whereas this mechanism assumed higher significance under EC at ambient +4°C temperature. We observed an enhancement in electron transport activity, photochemical potential, and net photosynthesis in spite of a loss in photostasis of photosynthesis under EC. On the other hand, the loss in photostasis of photosynthesis was exacerbated under EC at ambient +4°C temperature due to the decline in electron transport activity, photochemical potential, and net photosynthesis.

Additional key words: chlorophyll fluorescence; gas exchange; intrinsic water-use efficiency; lutein; violaxanthin; zeaxanthin.

Introduction

Alterations in structure and function of chloroplasts in response to increased atmospheric CO_2 concentration have been a matter of concern (Griffin *et al.* 2001, Zuo *et al.* 2002, Zhang *et al.* 2012). The elevated CO_2 concentration (EC) affects the developmental characteristics of the photosynthetic organelles in general (Nie *et al.* 1995, Miller *et al.* 1997). It induces a change in the volume density of thylakoids (Kutík *et al.* 1995) and increases the number and size of starch grains in chloroplasts (Zhang *et al.* 2012), besides increasing the ratio of stromal to granal thylakoids (Griffin *et al.* 2001). A higher number of plastoglobuli is observed in chloroplasts of leaves developed under EC (Velikova *et al.* 2009). CO_2 at a high concentration is known to improve the activities of antioxidant enzymes and it reduces the accumulation of malondialdehyde (MDA) (Zhang *et al.* 2012). Although contents of total chlorophyll (Chl) and Chl per unit fresh mass of leaf are reduced under EC, the rate of photosynthesis is increased (Zhang *et al.* 2012). Higher CO_2 concentration is also known to bring changes in the structure and composition of thylakoid membranes during development of the organelle (Robertson and Leech 1995).

High temperature, another major environmental stress factor, is also known to modulate the chloroplast functions (Joshi *et al.* 2013b, Mathur *et al.* 2014). The stress dismantles the thylakoid membrane, uncouples the light-harvesting complex II, reduces the oxygen evolution (Wen *et al.* 2005), and affects the activity of PSII (Pospíšil and

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Abbreviations: A – antheraxanthin; ACT – ambient CO_2 under ambient temperature; Car – carotenoids; Chl – chlorophyll; CS – cross section; DAE – days of the experiment; DI – dissipation; EC – elevated CO_2 concentration; ET – ambient temperature +4°C; F_m – maximum fluorescence; F_v – variable fluorescence; g_s – stomatal conductance; HPLC – high performance liquid chromatography; IPCC – Intergovernmental Panel for Climate Change; Lut – lutein; MDA – malondialdehyde; P_N – net photosynthetic rate; PSA – photosynthetic apparatus; ROS – reactive oxygen species; V – violaxanthin; WUE_i – intrinsic water-use efficiency; Z – zeaxanthin; β-Car – β-carotene.

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Tyystjärvi 1999) besides altering the thylakoid fluidity (Kim and Portis 2005). The electron transport chain in PSII is disrupted leading to the dissociation of antennae pigment-protein complexes from the reaction centres (Gounaris *et al.* 1984). On the other hand, enzymes of the Calvin-Benson cycle associated with PSI and structure of chloroplast envelope are not affected much by high temperature stress (Berry and Björkman 1980, Joshi *et al.* 2013b).

In nature, plants experience many environmental stress factors in combination and presently, a combination of stresses, EC and increased temperature, has become inevitable due to the climate change. According to the report of world meteorological organization, the global CO_2 concentration has increased over time to the current concentration of 390 ppm resulting in the global warming (IPCC Report 2001) and the concentration of CO_2 is expected to increase to 450 ppm by 2025 (IPCC Report 2007) resulting in a projected rise in global temperature of 1.4 to 5.8°C (IPCC Report 2014). These two environmental stresses (EC and increased temperature) in combination have been considered to be a major factor for limiting crop production in the future (Reddy and Gnanam 2000, Lobell *et al.* 2011). Therefore, the main objective of this study was to investigate the changes in structure and efficiency of photosynthetic apparatus of rice seedlings, a major crop plant and important cereal for human consumption in India, grown under EC and increased temperature environment in greenhouse conditions.

Materials and methods

Naturally ventilated polyhouse with a roof permanently covered with 200 µm UV-stabilizing polysheet and arrangements in order to maintain temperature and CO_2 concentration were constructed in the botanical garden of the college. Sides were permanently fixed with UV-stabilized, 40 mesh, anti-insect net and two separate curtains, one of polysheet apron of 0.92 m height on all four sides and one shade net with manual roll-up mechanism on two sides. The CO_2 concentration inside the polyhouse was maintained at either 390 ppm (current concentration; IPCC Report 2007) or 450 ppm (projected concentration for 2025; IPCC Report 2007) by adjusting the height of polysheet apron. The temperature inside the polyhouse was maintained by adjusting the height of the shade net and using the mist foggers and the exhaust fan fitted in the polyhouse. The temperature was maintained at either ambient (atmospheric; 30–31°C) or ambient +4°C (34–35°C; ET). The increase of 4°C in atmospheric temperature was within the projected rise of 1.4–5.8°C in global temperature from 1990–2100 with a harmful effect for food productivity (IPCC Report 2014).

Seeds of rice (*Oryza sativa* L. cv. Sri Ram 434, hybrid) were surface-sterilized with 30% ethanol and then kept in running water for six hours. The seeds were then rolled in wet blotting paper and kept in darkness for 24 h. The well germinated rice seeds were grown on sterilized cotton in Petri plates inside the polyhouse for a period of 18 d with natural day/night photoperiod under four experimental conditions:

	CO_2 concentration [ppm]	Temperature [°C]
ACT	390	30–31
ET	390	34–35
EC	450	30–31
EC + ET	450	34–35

The second leaf of rice seedlings, being the first complete leaf with a broad lamina exhibiting well differentiated phases and a longer duration of growth period, was suitable for different biophysical and biochemical measurements and therefore it was used as experimental materials in the present work.

Pigments were extracted from the second leaf of rice seedlings with prechilled 100% acetone. The extract was used for spectrophotometric determination (*UV-Vis spectrophotometer-117, Systronics,* India) of Chls and carotenoids (Car) as described by Wellburn and Lichtenthaler (1984). For high performance liquid chromatography (HPLC) analysis, the pigment extract from the second leaf of rice seedlings with 100% ice-cold acetone was centrifuged at 10,000 × *g* for 10 min. The filtered supernatant was used for high performance liquid chromatography (HPLC) analysis. Chromatography was carried out on a 3.91 × 150 mm *Nova-pak C18* analytical column (*Waters*, USA) following the method of de las Rivas *et al.* (1989). A fixed amount of the pigment extract was injected with *Waters 717 plus* autosampler. The column was equilibrated prior to injection of each sample by flushing with acetonitrile:methanol (mobile phase A) for 10 min. The elution solvent A contained acetonitrile and methanol in the ratio of 7:1 (v/v) and solvent B (mobile phase B) contained acetonitrile, methanol, water, and ethylacetate in the ratio of 7:0.96:0.04:5. Mobile phases were pumped by a *515 HPLC* pump (*Waters*, USA) at a flow rate of 0.3 ml min^{-1}. Pigments were identified by comparing their retention times with those of the standard. Peaks were scanned and detected by a *996 Photodiode Array Detector* integrated with *Waters Millennium* software (USA).

Accumulation of anthocyanin and flavonoids in the second leaf of 12-d-old rice seedlings was estimated according to the methods of Beggs and Wellmann (1994) and Flint *et al.* (1985), respectively.

The level of thylakoid lipid peroxidation in the second leaf of 12-d-old rice seedlings was measured as MDA accumulation following the method described by Panda *et al.* (1987).

Fluorescence transient and different fluorescence parameters were measured with a plant efficiency analyser (*Handy PEA*, *Hansatech*, UK) for the second leaf of rice seedlings according to Schreiber *et al.* (1986). The leaves were dark-adapted for 15 min and then exposed to a saturating pulse of red light for fluorescence measurements. The quantum yield of PSII photochemistry was calculated using the formula: $F_v/F_m = (F_m - F_0)/F_0$, where F_0 – minimal fluorescence of dark-adapted leaf; F_m – maximal fluorescence of dark-adapted leaf, and F_v – variable fluorescence (Schreiber *et al.* 1986, Strasser *et al.* 2000). PSII mediated electron transport activity was measured using the formula:

$$S_m/\tau_{fmax} = \frac{1}{\tau_{fmax}}\left[\int_0^{\tau_{fmax}}\{(F_m - F_t)\,dt\}\right]/(F_m - F_0);$$

where τ_{fmax} is the time to reach F_m (Strasser *et al.* 2000). The energy dissipation per excited cross section (DI/CS) was determined as: DI/CS = ABS/CS – TR/CS; where ABS/CS and TR/CS are phenomenological absorption flux and trapped flux for closure of reaction centre, respectively (Strasser *et al.* 2000).

The stomatal conductance (g_s) and the rate of CO_2 assimilation (P_N) were measured from the second leaf of rice seedlings at 10:00 h with a *PP System* (*Hansatech*, UK) fitted with a cuvette controlling the measuring conditions automatically. The temperature within the leaf chamber was maintained at 25°C. The rate of steady-state photosynthesis was measured at a saturating photon flux density of 1,600 μmol m^{-2} s^{-1} and a CO_2 supply of 375 μmol m^{-2} s^{-1} from a mini CO_2 cartridge. Intrinsic water-use efficiency (WUE_i) was calculated as the ratio P_N/g_s.

In order to assess the alteration in balance between energy source and energy sink (photostasis of photosynthesis), the ratio of (total Chl)/P_N was calculated (Joshi *et al.* 2013a).

Statistical analysis was carried out using *Student*'s *t*-test as described by Glantz (1989).

Results

In order to characterise the leaf developmental phases of the second leaf in the rice seedlings and to examine the effects of ET, EC, and EC + ET, the kinetics of total Chl and total Car contents were followed (Fig. 1). The total Chl content increased up to 11 d of the experiment (DAE), remained almost steady from 12 to 15 DAE, and declined thereafter. ET, however, suppressed the accumulation of total Chl but followed the same trend of the leaf development. Under EC, the Chl content increased up to 9 DAE, remained steady from 10 to15 DAE, and declined thereafter indicating the occurrence of changes in the phases of the leaf development. On the other hand, under EC + ET conditions, the Chl content increased up to 6 DAE, remained the same till 12 DAE, and thereafter it started declining. The peak values under ACT, ET, EC, and EC + ET conditions were 2.902 ± 0.013, 2.891 ± 0.032, 3.089 ± 0.017, and 2.931 ± 0.043 g(Chl) kg^{-1}(FM), respectively.

The total Car content in the second leaf of rice seedlings grown under ACT increased from 1 to 11 DAE, remained steady from 12 to 15 DAE, and then declined (Fig. 1*B*). In the seedlings grown under ET, the total Car content followed similar kinetics as that of the seedlings grown under ACT, but at a lower content. On the other hand, the Car content in the leaves grown under EC increased up to 8 DAE, remained almost steady from 9 to 18 DAE, while it increased under EC + ET up to 6 DAE, remained stable up to 12 DAE, and declined thereafter. The peak values of Car under ACT, ET, EC, and EC + ET were 0.512 ± 0.033, 0.477 ± 0.019, 0.524 ± 0.022, and 0.469 ± 0.027 g(Car) kg^{-1}(FM), respectively.

In order to verify the phases of growth in terms of photochemical potential under the experimental conditions, the kinetic of changes in F_v/F_m from the Chl *a* fluorescence transients was followed in the second leaf of the rice seedlings (Fig.1*C*). The patterns of changes in F_v/F_m under these conditions were almost similar to the patterns of changes in both Chl and Car.

The relative changes in the total Chl and Car contents in the second leaf of rice seedlings grown under ACT, ET, EC, and EC + ET after 12 DAE are depicted in Fig. 2*A*,*B*, respectively. The Chl content decreased by 1.1 and 0.6% in the seedlings grown under ET and EC + ET, respectively, while it increased by 8.5% in the seedlings grown under EC conditions. On the other hand, the Car content increased under EC by 2.1% and decreased under ET and EC + ET by 13.7 and 19.7% , respectively.

In order to examine the relative stability of Chl *a*, Chl *b*, and Car in the second leaf of 12-d-old rice seedlings grown under ET, EC, and EC + ET, the ratios of Chl/Car (Fig. 2*C*) and Chl *a*/*b* (Fig. 2*D*) were compared with that of the seedlings grown under ACT. The ratio of Chl/Car increased by 14.6, 6, and 23%, while the ratio of Chl *a*/*b* decreased by 1.3, 6.2, and 8.8% under ET, EC, and EC + ET, respectively.

The changes in the contents of β-Car and Lut in the second leaves of 12-d-old rice seedlings grown under ACT, ET, EC, and EC + ET conditions are described in Table 1. The contents of β-Car and Lut increased under all conditions. The amount of β-Car increased by 2.1, 2.8, and 5.7%, while Lut increased by 9.4, 34.5, and 39.8% in the samples grown under ET, EC, and EC + ET conditions, respectively.

The relative changes in the content of flavonoids, anthocyanin, and MDA in the second leaf of rice seedlings grown under ET, EC, and EC + ET conditions are also described in Table 1. The amount of flavonoids increased marginally by 0.6% and significantly by 13.9% in the second leaf of rice seedlings grown under EC and EC + ET, respectively, while the parameter decreased by

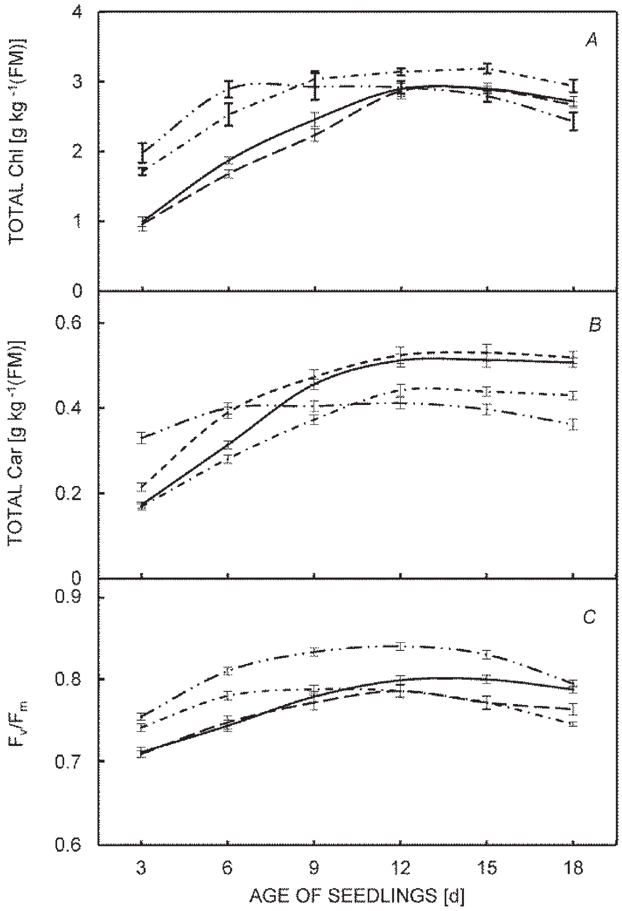

Fig. 1. Changes in the total chlorophyll (Chl) content (*A*), total carotenoid (Car) content (*B*), and ratio of variable fluorescence to maximum fluorescence (F_v/F_m) (*C*) in the second leaves of rice seedlings for description of different phases of growth and development under ambient (——), ET (- - -), EC (– · – · –), and EC + ET (– ·· – ·· –). Each value is a mean of three independent sets of experiments with five replicates in each set. Bar indicates ± SD.

1.6% in the seedlings grown under ET. The anthocyanin content increased by 7.1, 40.2, and 26.3% in the samples grown under ET, EC, and EC + ET, respectively. The accumulation of MDA, however, was elevated by 1.2 and 5.6% in the samples grown under ET and EC + ET condition, while it declined marginally by 1.3% under EC. The alteration in the content of different components of xanthophyll cycle, as obtained from HPLC analyses are described in Table 2. In the leaves grown under ET, the contents of violaxanthin (V) and antheraxanthin (A) increased marginally at the expense of zeaxanthin (Z) so that the V/Z increased by 47.7% and (A+Z)/(V+A+Z) declined by 5.1%. On the other hand, the content of Z increased by 62% at the expense of V and A, so that the V/Z ratio decreased by 55.5% and (A+Z)/(V+A+Z) ratio increased by 5.0% in the leaves grown under EC. Under EC + ET, the V/Z ratio increased by 250% and the ratio of (Z+A)/(V+Z+A) decreased by 14.5% although the concentration of A remained almost unchanged.

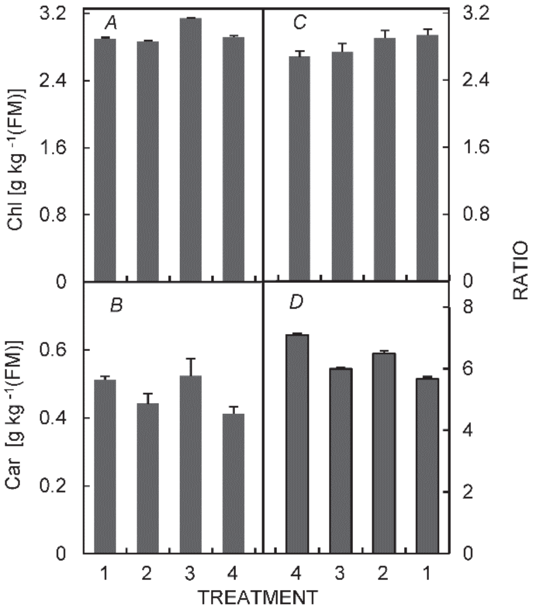

Fig. 2. Histograms showing changes in the content of total Chl (*A*), total Car (*B*), Chl/Car (*C*), and Chl *a*/*b* (*D*) in the second leaf of 12-d-old rice seedlings grown under ambient (1), ambient +4°C (ET) (2), elevated carbon dioxide (EC) (3), and elevated carbon dioxide +4°C (EC + ET) (4) conditions. The levels of statistical significance under ET, EC, and EC + ET (difference from the values in sample grown under ambient condition) are ($p<0.05$), ($p<0.05$), and ($p<0.5$) for Chl; ($p<0.001$), ($p<0.1$), and ($p<0.001$) for Car; ($p<0.005$), ($p<0.001$), and ($p<0.005$) for Chl/Car; and ($p<0.5$), ($p<0.05$), and ($p<0.001$) for Chl *a*/*b*, respectively. Each value is a mean of three independent set of experiments with five replicates in each set. Bar indicates ± SD.

Table 1. Changes in the contents of lutein (Lut), β-carotene (β-Car), and accumulation of flavonoids, anthocyanin, and malondialdehyde (MDA) in the second leaf of 12-d-old rice seedlings grown under ambient, ambient +4°C (ET), elevated CO_2 concentration (EC), and EC + ET conditions. 100% of Lut, β-Car, flavonoid, anthocyanin and MDA represent 4.26 [mmol g^{-1}(FM)], 258 [mmol g^{-1}(FM)], 15.274 [A_{270} g^{-1}(FM)], 2.208 [A_{546} g^{-1}(FM)] and 0.34 [nmol g^{-1}(FM)], respectively. Each value is a mean of three independent experiments. The levels of statistical significance (difference from the values in sample grown under ambient condition) are indicated by asterisks *, **, ***, and **** representing ($p<0.005$), ($p<0.05$), ($p<0.01$), and ($p<0.1$), respectively.

Parameter	Ambient	ET	EC	EC + ET
Lut	100	109.4^{**}	134.4^{*}	139.8^{*}
β-Car	100	102.1^{***}	102.8^{***}	105.7^{**}
Flavonoids	100	98.4^{***}	100.4^{****}	113.4^{**}
Anthocyanin	100	107.1^{**}	140.3^{*}	126.2^{*}
MDA	100	101.2^{****}	98.8^{****}	106.5^{***}

Table 2. Relative changes in the content of different components of xanthophyll cycle, *i.e.*, violaxanthin (V), antheraxanthin (A), and zeaxanthin (Z) in the second leaf of 12-d-old rice seedlings grown under ambient temperature, ambient +4°C (ET), elevated CO_2 concentration (EC), and EC + ET. The values are calculated as the percentage of total xanthophyll pool after normalising against the total Chl content. Each value is a mean of three independent experiments. The levels of statistical significance (difference from the values in the sample grown under ambient condition) are indicated by asterisks *, **, and *** representing ($p<0.005$), ($p<0.05$), and ($p<0.1$), respectively.

Parameter	Ambient	ET	EC	EC + ET
V	15.7	20.4^*	11.7^*	28.4^*
A	40.3	42.1^{**}	16.8^*	40.3^{***}
Z	43.5	37.4^*	70.5^*	31.3^*
V/Z	0.369	0.545^*	0.166^{**}	0.905^*
(A+Z)/(V+A+Z)	0.839	0.796^{**}	0.882^{**}	0.717^{**}

The changes in different parameters of Chl *a* fluorescence are described in Table 3. The measure of the maximum yield of primary photochemistry (F_v/F_m) increased by 0.9, 5.3, and 1.4% under ET, EC, and EC + ET, respectively. The results of fluorescence transient parameters (*1*) the whole chain electron transfer rate (S_m/τ_{fmax}) and (*2*) dissipation per excited cross section (DI/CS) are also described in Table 3. The parameters S_m/τ_{fmax} and DI/CS declined by 0.7 and 0.3%, respectively, in sample grown under ET. On the other hand, the parameter Sm/τ_{fmax} increased by 3% and decreased by 15.2%, while DI/CS decreased by 15 and 8.3% under EC and EC + ET, respectively.

The changes in P_N, g_s, and WUE_i in the second leaf of rice seedlings grown under ACT, ET, EC, and EC + ET conditions are also described in Table 3. The value of P_N increased by 5.4% under EC, while it decreased by 1.4 and 3.3% under ET and EC + ET conditions, respectively. The value of g_s decreased by 18% under EC and increased by 8 and 24% under ET and EC + ET growth conditions, respectively. On the contrary, WUE_i increased by 29% under EC, while the parameter decreased by 8.0 and 21.7% in the seedlings grown under ET and EC + ET conditions.

The ratio of (total Chl)/P_N increased by 0.3, 3.2, and 4.3% under ET, EC, and EC + ET, respectively.

Table 3. Changes in Chl *a* fluorescence transient parameters as measured with a *PEA* and gas-exchange parameters as measured with a *PPS* from the second leaf of 12-d-old rice seedlings grown under ambient temperature, ambient +4°C (ET), elevated CO_2 concentration (EC), and EC + ET environments. Intrinsic water-use efficiency (WUE_i: P_N/g_s) and (total Chl)/P_N were calculated. Each value is a mean ± SD of four independent set of experiments with five replicates in each set. The levels of statistical significance (difference from the values in sample grown under ambient condition) are indicated by asterisks *, **, and *** representing ($p<0.005$), ($p<0.05$), and ($p<0.1$), respectively.

Parameter	Ambient	ET	EC	EC + ET
F_v/F_m	0.751 ± 0.007	0.757 ± 0.003	0.789 ± 0.004	0.76 ± 0.01
$\mathbf{S_m/}\tau_{\mathbf{fmax}}$ [barn/ms]	0.0099	0.0096^{**}	0.0102^*	0.0084^*
DI/CS	27.63	27.54^{***}	23.404^{**}	25.33^{**}
P_N [μmol m^{-2} s^{-1}]	9.2 ± 1.3	9.07 ± 0.97	9.70 ± 1.08	8.9 ± 2.1
g_s [mmol(H_2O) m^{-2} s^{-1}]	0.5 ± 0.08	0.54 ± 0.03	0.41 ± 0.14	0.62 ± 0.16
WUE_i [mol(CO_2) mol(H_2O)$^{-1}$]	0.0184 ± 0.0020	0.0168 ± 0.0068	0.0237 ± 0.0041	0.0144 ± 0.0048
(Total Chl)/P_N [%]	100	100.3	103.2^{**}	104.3^{**}

Discussion

The courses of the total Chl content, total Car content, and photochemical potential of the second leaf of rice seedlings were used to characterise different phases of leaf growth and development. The pattern of changes in the content of total Chl (Fig. 1*A*) and total Car (Fig. 1*B*) grown under ambient conditions demonstrated the occurrence of the developmental phase from 1 to 11 DAE, steady phase from 12 to 15 DAE, and senescence thereafter. It was also supported by the course of the photochemical potential (F_v/F_m, Fig. 1*C*). The second leaves of the rice seedlings grown under EC and EC + ET, however, experienced a different growth pattern with temporal shifts in the course of their development. The results were in agreement with the observation of Miller *et al.* (1997). Our results suggest that the steady phase of leaf development in the seedlings grown under EC occurred earlier and the duration of the phase was prolonged. The results in Fig. 1 demonstrated that on 12 DAE, the second leaves of rice seedlings experienced the steady phase of development under all the experimental conditions examined in this work, and therefore all the parameters measured from the second leaves of 12-d-old rice seedlings were compared in order to examine the changes in photosynthetic responses under these conditions.

We observed the enhancement in both Chl and Car content in response to EC, in contrast to the findings of Nakano *et al.* (1997) and Kim and You (2010). Reports demonstrating variation in the content of total Chl and total Car in response to CO_2 enrichment in different plant systems are available in literature (Stylinski *et al.* 2000, Rasineni *et al.* 2011). The increase in the pigment contents under the EC conditions could be caused by a decline in light intensity inside the polyhouse. The increase in the total Chl content (Fig. 2*A*), total Car content (Fig. 2*B*), Chl/Car ratio (Fig. 2*C*), and the decrease in the Chl *a*/*b* ratio (Fig. 2*D*) in response to EC suggested that CO_2 enrichment affected photosynthetic apparatus at the level of structural organisation. Additionally, the relatively higher content of total Chl as compared to total Car suggests that the photosynthetic apparatus (PSA) did not face any challenge from oxidative damage. On the other hand, the relatively lower Car content as compared to Chl (higher Chl/Car ratio) (Fig. 2*C*) in the plants grown under ET or EC + ET compared with the corresponding values in the plants grown under ACT or EC, respectively, may render the PSA susceptible to temperature-mediated oxidative stress.

Car is known to protect the PSA from oxidative damage and help in dissipating harmful quanta (Demmig-Adams 1990). While β-Car is involved in molecular defence system (Mohr and Schopfer 1995), the xanthophyll cycle is implicated in the radiationless decay of unused light quanta (Gilmore 1997, Havaux and Niyogi 1999). From our results (Tables 1, 2) and higher P_N (Table 3), it appeared that EC alleviated the formation of reactive oxygen species (ROS), by channelling more electrons into carbon-fixation pathways and less electrons into ROS-producing pathways (Asada 1999). Previous studies addressing oxidative stress and antioxidative defence responses demonstrate that plants grown under EC have decreased photooxidative stress compared to ones grown under normal CO_2 concentration (Aranjuelo *et al.* 2008). On the other hand, under EC + ET, the increase in the oxidative defence system become evident from the results of Lut, β-Car (Table 1), and components of the xanthophyll cycle (Table 2). However, the impact of increased temperature (by 4°C) was meagre under ambient condition. Additionally, Lut plays a fundamental role in the assembly and development of PSA (Jahns and Holzwarth 2012) and the increase in the content of Lut in the plants grown under EC and EC + ET could be a developmental strategy.

The xanthophyll cycle plays a photoprotective role for PSA and the protection is higher when the deepoxidation state of the xanthophyll cycle [(A+Z)/(V+A+Z)] is higher (Gilmore 1997, Havaux and Niyogi 1999). The contents of both A and Z increased while that of V declined resulting in an increase in the ratio of (A+Z)/(V+A+Z) in leaves of the seedlings grown under EC (Table 2). The increase in this ratio implies that the rate of conversion of different states of xanthophylls increased. The results are in conformity with the findings of Aranjuelo *et al.* (2008) and may be suggestive of enhanced photoprotection *via* thermal dissipation of excitation energy and avoidance of ROS formation (Murchie and Niyogi 2011). A higher content of Z is known to facilitate the energy flow amongst the xanthophylls through a molecular gearshift mechanism (Frank *et al.* 1994). The increase in the Z value under EC as inferred from the V/Z ratio (Table 2) is suggestive of its role in energy dissipation. However, the capacity was diminished significantly under EC + ET. Almost equal amount of both V and Z components of the xanthophyll cycle in the leaves of seedlings grown under EC + ET suggested an impairment of the cycle, which was reflected by the MDA accumulation (Table 1).

The content of MDA is a measure of peroxidation of membrane lipids in plants exposed to different abiotic stress factors (Gülen *et al.* 2008), while flavonoids are known for their antioxidant property (Mohr and Schopfer 1995). We did not observe any significant changes in the amounts of either MDA or flavonoids in the second leaf of rice seedlings grown under EC. These results strengthen the belief that the seedlings grown under EC did not face any oxidative stress. On the other hand, anthocyanin and flavonoids are known to protect DNA (Stapleton and Walbot 1994) and their enhancement in EC + ET plants (Table 1) could be an adaptive strategy against the temperature-mediated oxidative stress.

In order to examine the effect of EC with or without ET on photosynthetic capacity, we followed the Chl *a* fluorescence kinetics. The Chl *a* fluorescence transient measurement is a rapid, noninvasive tool to monitor and characterize different photochemical events (Schreiber *et al.* 1986, Strasser *et al.* 2000). Electron transport through PSII (whole chain electron transport) as measured in term of S_m/τ_{fmax} (Table 3) showed a marginal enhancement under EC, but the decline of the parameter under EC + ET suggested an impairment in the electron transport chain. Further, the decline in DI/CS demonstrated a decline in dissipation of energy in the form of heat under both these environmental conditions which was indicative of a lesser role of the photoprotective mechanism.

We observed an increase in photosynthetic efficiency (F_v/F_m) under all the experimental conditions. The high F_v/F_m values observed in dark-adapted leaves of both EC and EC + ET plants (Table 3) indicated that these conditions did not impose any photoinhibition (Baker 2008); it corroborated with our view of zero oxidative stress. Under ambient environment and optimal water conditions, the values of both CO_2 assimilation and g_s were quite similar to that in tomato plants (Giorio *et al.* 2012) confirming the absence of any stress during the growth of rice seedlings (Table 3). The decline in g_s under EC (Table 3) in this work was in agreement with findings of Ainsworth and Rogers (2007). The increases in P_N and F_v/F_m in spite of decline in g_s under EC (Table 3) suggested improvement in the functioning of PSA and the ameliorating role of WUE_i (Table 3). The increase in the ratio of (total Chl)/P_N, indicating a loss in photostasis of photosynthesis (Joshi *et al.* 2011, 2013a), might suggest that the carbon assimilation process was affected relatively more than the energy absorption process under EC and high temperature exacerbated the effect under EC + ET conditions. These results could be correlated to the loss in the rate of electron transport between PSII and PSI under EC + ET as mentioned earlier.

We, therefore, believe that the enhancement in the atmospheric CO_2 concentration, in spite of its effect on leaf growth and development, may not be detrimental to photosynthesis of rice seedlings in near future, but the concomitant rise in temperature might have an alarming impact.

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