

# Estimation of photorespiration rate by simultaneous measurements of CO<sub>2</sub>, gas exchange rate, and chlorophyll fluorescence quenching in the C<sub>3</sub> plant *Vigna radiata* (L.) Wilczek and the C<sub>4</sub> plant *Amaranthus mongostanus* L.

Y. YOSHIMURA\*, F. KUBOTA\*\*, and K. HIRAO\*\*

Graduate School of Bioresource and Bioenvironmental Sciences\* and Faculty of Agriculture\*\*,  
Kyushu University, Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan

## Abstract

So far the photorespiration rate ( $R_p$ ) in a leaf has been determined as the difference between the net photosynthetic rates ( $P_N$ ) measured in 21 % O<sub>2</sub> air ( $P_{N21\%}$ ) and 3 % O<sub>2</sub> air ( $P_{N3\%}$ ). In the C<sub>3</sub> plant *Vigna radiata* and the C<sub>4</sub> plant *Amaranthus mongostanus* L.,  $P_N$  and chlorophyll fluorescence quenching in leaves were monitored simultaneously.  $R_p$  of leaves *in situ* was estimated as termed  $R_{PE}$  from the electron transport rates through photosystem 2 (PS2), and compared with  $R_{PO}$  ( $P_{N3\%} - P_{N21\%}$ ). In *V. radiata*  $R_{PO}$  was 11.9  $\mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$  and the ratio of  $R_{PO}$  to  $P_{N21\%}$  was 42.2 %, whereas the ratio of  $R_{PE}$  to  $P_{N21\%}$  was 25.7 %. This suggests that  $R_{PO}$  may be over-estimated for the real  $R_p$  in normal air. In *A. mongostanus*,  $P_N$  was almost not changed with a decrease in O<sub>2</sub> concentration from 21 to 3 %, whereas the quantum yield of PS2 was evidently affected by the change in O<sub>2</sub> concentration. This fact shows the presence of photorespiration in this C<sub>4</sub> species, where  $R_{PE}$  was equivalent to 3.8 % of  $P_{N21\%}$ .

*Additional key words:* electron transport rate; photosynthesis; photosystem 2; mungbean; quantum yield.

## Introduction

Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) has a dual function as carboxylase in carbon assimilation and also as oxygenase in photorespiration. Its functional characteristics in leaf vary with the relative concentrations of CO<sub>2</sub> and O<sub>2</sub> in ambient air. The intercellular CO<sub>2</sub> concentration in leaves growing under field conditions greatly changes with stomata aperture, which may often give a significant effect to both CO<sub>2</sub> assimilation rate and photorespiration rate in various ways depending on other environmental factors such as air temperature, moisture, and photosynthetic photon flux density (PPFD).

Photorespiration has often been regarded as a negative function in biomass production, because a considerable part of CO<sub>2</sub> photosynthetically fixed in a leaf is released to the atmosphere through C<sub>2</sub> cycle. On the other hand,

photorespiration protects photosynthetic and other physiological functions in plant by dissipating the chemical energy excessively produced by photochemical systems. This functional importance is more accentuated when plants, particularly C<sub>3</sub> plants, are subjected to stresses such as high irradiance and water deficits (Heber *et al.* 1996, Kozaki and Takeba 1996).

So far the photorespiration rate ( $R_p$ ) in C<sub>3</sub> plants has been usually determined and evaluated as the value obtained by subtracting  $P_{N21\%}$  from  $P_{N3\%}$ . However, some methodological defects have been pointed out for this method. Under a low O<sub>2</sub> concentration, such as 3 % in air, the activity of oxygenase in RuBPCO is restricted, but the carboxylase activity is enhanced instead. Hence, the subtracted value is an over-estimated value compared to the real  $R_p$  in 21 % O<sub>2</sub> air (deVeau and Burris 1989, Tokuda

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*Abbreviations:* Chl – chlorophyll;  $F_m'$  – maximum fluorescence in light;  $F_t$  – steady-state fluorescence in light-adapted state;  $g_s$  – stomatal conductance;  $J_T$  – the rate of electron transport through photosystem 2;  $k_c$  – the number of electron equivalents required to reduce 1 molecule CO<sub>2</sub> in 3 % O<sub>2</sub> air;  $k_N$  – the number of electron equivalents required to reduce 1 molecule CO<sub>2</sub> in 21 % O<sub>2</sub> air;  $k_r$  – the number of electron equivalents required to release 1 molecule CO<sub>2</sub> in photorespiration; NAD-ME – NAD-malic enzyme;  $P_G$  – gross photosynthetic rate,  $P_G = P_N + R_D$ ;  $P_N$  – net photosynthetic rate;  $P_{N21\%}$  – net photosynthetic rate measured in 21 % O<sub>2</sub> air;  $P_{N3\%}$  – net photosynthetic rate measured in 3 % O<sub>2</sub> air;  $R_p$  – photorespiration rate;  $R_{PE}$  – photorespiration rate estimated by energetic calculation;  $R_{PO}$  – photorespiration rate estimated by subtracting  $P_{N21\%}$  from  $P_{N3\%}$ ; PPFD – photosynthetic photon flux density; PS – photosystem;  $R_D$  – dark respiration rate; RuBPCO – ribulose-1,5-bisphosphate carboxylase/oxygenase;  $\Delta\text{CO}_2$  – the difference of CO<sub>2</sub> concentration between sample air and reference air which flow through the leaf chamber;  $\Delta\text{H}_2\text{O}$  – the difference in H<sub>2</sub>O vapour pressure between sample air and reference air which flow through the leaf chamber;  $\Phi_e$  – quantum yield of photosystem 2.

*et al.* 1999). In order to know  $R_p$  that is more accurate or closer to the real value, it is essential to determine it in normal air containing 21 %  $O_2$ .

In general, photorespiration in  $C_4$  plants is almost completely depressed because malate and aspartate produced in mesophyll cells at the initial reaction of photosynthesis in  $C_4$  plants provide highly concentrated  $CO_2$  to RuBPCO in the bundle sheath cells (Edwards and Walker 1983, Percy and Ehleringer 1984, Edwards *et al.* 1985). However, the existence of photorespiration is known in  $C_4$  species by observing the incorporation of  $^{18}O_2$  into metabolites formed as a consequence of photorespiration (Berry *et al.* 1978, deVeau and Burris 1989). Maroco *et al.* (1997, 2000) showed that the  $CO_2$  assimilation rate in three subtypes of  $C_4$  plants increased at an optimal  $O_2$  concentration of 5 to 10 %. This may indicate that  $C_4$  plants conduct a considerable photorespiration depending on the environment. But gas exchange measurements are inadequate as a method to detect slight differences between the  $P_N$  in  $C_4$  plant observed at 3 and 21 %  $O_2$ .

Recently many studies have been performed on the

chemical energy sharing between  $CO_2$  assimilation and other metabolisms (photorespiration and Mehler reaction), using the chlorophyll (Chl) fluorescence quenching diagnosis (Di Marco *et al.* 1994, Di Martino *et al.* 1999, Tsuyama and Kobayashi 1999, Miyake and Yokota 2000, Muraoka *et al.* 2000, Ruuska *et al.* 2000). Nevertheless, not much information is available on  $R_p$  and the relation of  $R_p$  to  $P_{N21\%}$  in crop leaves evaluated by Chl fluorescence diagnosis and their specific and cultivar characteristics. To know real values of  $R_p$  in crop leaves, it is essential to deepen the understanding of the role of photorespiration as a function necessary for growth and productivity maintenance of the plant.

In this study we examined how to estimate a more correct  $R_p$  of leaves *in situ* based on the changing electron transport rates by a simultaneous monitoring of  $CO_2$  exchange rate and Chl fluorescence quenching. The  $P_N$  and  $R_p$  were calculated and compared between the  $C_4$  plant, *Amaranthus mongostanus*, and the  $C_3$  plant, *Vigna radiata*.

## Materials and methods

The  $C_3$  plant mungbean, *V. radiata* (L.) Wilczek cv. Chinese and the  $C_4$  plant *A. mongostanus* L. were grown in 8 500 cm<sup>3</sup> pots (two plants per pot for *V. radiata*, one plant per pot for *A. mongostanus*) filled with sandy soil containing a chemical compound fertiliser (N, P, and K each 0.8 g per pot) during summer season in the experimental field of Kyushu University (33°35'N, 130°23'E).

Gas exchange and Chl fluorescence were simultaneously measured with full expanded, attached leaves of both species.  $P_N$ ,  $R_D$ , and transpiration rate were measured with a sandwich-type assimilation chamber (PLD-B, ADC, Hoddesdon, UK) in an open gas exchange system. Sampled air was monitored with an infrared  $CO_2$  analyser (LI6262, Li-Cor, Lincoln, USA). The  $O_2$  concentration in air pumped to the leaf chamber was alternatively shifted 3 times between 21 and 3 %  $O_2$  for about 100 min. The air containing 350  $\mu\text{mol}(CO_2) \text{ mol}^{-1}$  and 3 or 21 %  $O_2$  was prepared by mixing  $N_2$ ,  $O_2$ , and  $CO_2$  with a gas mixer (GM-3A, KOFLOC, Kyoto, Japan).  $CO_2$  absorbent (soda lime) was used for the final adjustment of  $CO_2$  concentration. The air had been moisture-saturated at 18.3 °C by a dew point generator (LI610, Li-Cor, Lincoln, USA) before it was sent to the assimilation chamber, and the air flow rate through the chamber was 16.67 cm<sup>3</sup> s<sup>-1</sup>. Leaf temperature was adjusted to 30.2 ± 0.6 °C by circulating temperature-controlled water to the radiator attached to the chamber. A metal halogen lamp (HILUX-HR, Rikagaku Co., Japan) provided actinic irradiation of 1 600  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  PPFD.

The quantum yield of PS2 ( $\Phi_e$ ) was measured with a Chl fluorometer (PAM-2000, Walz, Effeltrich, Germany),

and the head of the fluorescence probe guide was fixed on the assimilation chamber at the position where it did not shade the leaf.  $\Phi_e$  was calculated from the Eq. (1) proposed by Genty *et al.* (1989):

$$\Phi_e = (F'_m - F_t)/F'_m \quad (1)$$

where  $F_t$  is Chl fluorescence emitted by leaf, measured at the steady state, and  $F'_m$  is the fluorescence spike shown by giving 1.2 s pulse of photosynthesis saturating irradiation of 8 000  $\mu\text{mol m}^{-2} \text{ s}^{-1}$  PPFD.

The rate of electron transport through PS2 ( $J_T$ ) was calculated from Eq. (2):

$$J_T = \Phi_e L \times 0.5 \times a \quad (2)$$

where  $L$  is the PPFD at leaf surface (1 600  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ). Assuming that the photons are evenly distributed to the two photosystems, 0.5 is used in Eq. (2).  $a$  is the ratio of Chl-absorbed photons to the incident photons. Values of  $a$ , 0.935 and 0.921, were determined with *V. radiata* and *A. mongostanus*, respectively.

The parameter  $k_c$  is the number of electron equivalents required to reduce 1 molecule of  $CO_2$  in the Calvin cycle. The theoretical minimum value of  $k_c$  is 4, but in our study its value was calculated from Eq. (3) using the parameters measured without photorespiration in 3 %  $O_2$  air.  $k_c$  determined in 21 %  $O_2$  air is termed  $k_N$ .

$$k_c = J_T/P_G \quad (3)$$

where  $P_G$  is a sum of  $P_N + R_D$ . The Eq. (3) is rewritten as Eq. (4) using parameters of RuBP carboxylation rate ( $V_c$ ) and RuBP oxygenation rate ( $V_o$ ), and then Eq. (5) is deduced:

$$P_G = V_c - 0.5 V_0 \quad (4)$$

$$J_T = k_c V_c + 0.5 k_r V_0 \quad (5)$$

where  $k_r$  is the number of electron equivalents required to release 1 molecule of  $\text{CO}_2$  in photorespiration. The equation  $J_T = 4 V_c + 4 V_0$  proposed by Farquhar *et al.* (1980) has been frequently used in recent studies (Di Martino *et al.* 1999, Miyake and Yokota 2000, Muraoka *et al.* 2000). The number of electrons required to fixing 1 molecule  $\text{O}_2$  in the  $\text{C}_2$  cycle is between 4 and 6. For instance, Di Marco *et al.* (1994) used the equation of  $J_T = 4 V_c + 6 V_0$ . We used the experimentally determined value as coefficients in Eq. (5).

$k_r$  is estimated from the Eq. (6) that is based on the ratio of 18.5 ATP and 9 ATP, both of which are chemically equivalent energies consumed for release of 1 molecule  $\text{CO}_2$  in  $\text{C}_2$  cycle and fixation of 1 molecule  $\text{CO}_2$  in  $\text{C}_3$  cycle, respectively:

$$k_r = k_c 18.5/9.0 \quad (6)$$

In  $\text{C}_4$  plants,  $k_r$  is calculated from Eq. (7) because the  $\text{CO}_2$  concentrating mechanism requires additional 2 ATP to convert pyruvate to phosphoenolpyruvate in each turn of the  $\text{C}_4$  cycle in the NAD-malic enzyme (NAD-ME) subtype such as *A. mongostanus* (Table 1):

$$k_r = k_c 18.5/11.0 \quad (7)$$

When the values of  $J_T$ ,  $k_c$ ,  $V_c$ , or  $\text{CO}_2$  assimilation rate measured in 3 %  $\text{O}_2$  air and  $k_r$  are substituted in the Eq. (5),  $R_p$  can be obtained from Eq. (8):

$$R_{PE} = 0.5 V_0 \quad (8)$$

The photorespiration ratio based on chemical energy is written as  $R_{PE}/P_{N21\%}$ , where  $P_{N21\%}$  is a value measured in 21 %  $\text{O}_2$  air. The  $R_p$  determined by subtracting  $P_{N21\%}$  from  $P_{N3\%}$  is termed  $R_{PO}$  here, and in this case the photorespiration ratio is  $R_{PO}/P_{N21\%}$ .

## Results and discussion

Table 1 shows the values of  $k_c$  given theoretically and  $k_r$  determined experimentally. The theoretical minimum value of  $k_c$  is 4.00, and that of NAD-ME subtype is 4.89. It is a little larger than 4.00, because the chemical energy required for fixing 1 molecule of  $\text{CO}_2$  in this subtype is equivalent to 11 molecules of ATP compared to 9 ATP molecules in  $\text{C}_3$  plants. The theoretical value of  $k_r$ , 8.22, is more than two-fold the  $k_c$  value because the energy used for releasing 1 molecule  $\text{CO}_2$  in photorespiration is equivalent to 18.5 ATP.

The experimentally determined  $k_c$  value in *V. radiata* obtained by Eq. (3) was  $4.63 \pm 0.12$  as shown in Table 1, which was higher than the theoretical minimum value of 4.00. According to Björkman and Demmig (1987) the mean value of maximum quantum yields for 37  $\text{C}_3$  species was 0.106 ( $\text{O}_2$  intake/photon absorbed), which corresponds to  $k_c = 4.72$  that is calculated on the basis of 1  $\text{CO}_2$  fixation in  $\text{C}_3$  cycle. Using a peeled leaf of *V. radiata* without gas exchange restriction, Tokuda *et al.* (1999) obtained 4.62 for  $k_c$ , which is close to the value determined here.

In the  $\text{C}_3$  plant *V. radiata*, the difference of  $\text{CO}_2$  concentration ( $\Delta\text{CO}_2$ ) between sample air and reference air showed clear oscillations according to the oxygen concentration change in the air flown to the leaf chamber (Fig. 1). These oscillations mean that  $P_G$  increased with a change in  $\text{O}_2$  concentration from 21 to 3 %. In contrast to this,  $\Phi_e$  showed a counter directional change with change in  $P_G$  (Fig. 1). The difference of  $\text{H}_2\text{O}$  vapour pressure ( $\Delta\text{H}_2\text{O}$ ) between sample air and reference air was almost constant. This means that stomatal conductances ( $g_s$ ) were not significantly different between the measurements in 21 and 3 %  $\text{O}_2$ .  $g_s$  was about  $0.3 \text{ mmol m}^{-2} \text{ s}^{-1}$  (Table 2). This  $g_s$  is sufficient to perform gas exchange between the ambient air and the leaf of *V. radiata*.

Table 1. The experimental values of electron equivalent to reduce or release in photorespiration 1  $\text{CO}_2$  molecule in *V. radiata* and *A. mongostanus* and the theoretical minimum values in the Calvin cycle and  $\text{C}_4$  cycle of NAD-ME type. Means  $\pm$  SE of three replications.  $k_c$  = the number of electron equivalents to reduce 1  $\text{CO}_2$  molecule in the Calvin cycle.  $k_r$  = the number of electron equivalents to release 1  $\text{CO}_2$  molecule in photorespiration.

	Theoretical minimum value		Experimental value	
	$\text{C}_3$	$\text{C}_4$	<i>V. radiata</i> ( $\text{C}_3$ )	<i>A. mongostanus</i> ( $\text{C}_4$ )
$k_c$	4.00	4.89	$4.63 \pm 0.12$	$5.05 \pm 0.22$
$k_r$	8.22	8.22	9.52	8.49

Zelitch (1971) reported that photosynthetic rate increased by 33 to 50 % in  $\text{C}_3$  plants when  $\text{O}_2$  concentration of the ambient air decreased from 21 % to 3 or 1 %. Akita (1980) also reported that such an increase in  $P_N$  of 40 rice cultivars was 32.5 % and that in 6 dicotyledonous plants it was 40.8 % at a leaf temperature of 30 °C and irradiance of  $1450 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . As shown in Fig. 1 and Table 2, the increase in  $P_N$  of *V. radiata* by restricting photorespiration in 3 %  $\text{O}_2$  air was about 42 %, which was almost similar to the results of Zelitch and Akita.

$R_{PE}$  in *V. radiata* was  $7.3 \pm 0.4 \mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$  (Table 2). It was lower than  $R_{PO}$  [ $11.9 \pm 0.2 \mu\text{mol}(\text{CO}_2) \text{ m}^{-2} \text{ s}^{-1}$ ].  $R_{PE}/P_{N21\%}$  was  $25.7 \pm 0.9 \%$ , lower than  $R_{PO}/P_{N21\%}$  ( $42.2 \pm 0.9 \%$ ). In wheat, DeVea and Burris (1989) estimated  $R_p$  using the rate of incorporation of  $^{18}\text{O}_2$  into glycylate. They estimated a photorespiration ratio of 26.9 %, which is similar to the value of  $25.7 \pm 0.9 \%$  obtained here in *V. radiata*.  $R_{PO}$  is inevitably an overestimate of the real  $R_p$  performed in the normal air. Under the non-photorespiratory condition such as in air with

3 % O<sub>2</sub>, RuBP is not consumed in photorespiration and accumulated in leaf, and this may result in a significant promotion of the carboxylase activity and CO<sub>2</sub> assimilation.

The value of  $k_N$  was 8.03 (Table 2), which was about twice the theoretical minimum value ( $k_c = 4$ ) due to the additional requirement of chemical energy by photorespiration.  $J_T/P_N$  was 8.6, which was close to the range 8.7 to 10.8 obtained by Krall and Edwards (1992) in wheat under ambient air.

In the C<sub>4</sub> plant *A. mongostanus*, the change in O<sub>2</sub> concentration in the leaf chamber from 21 to 3 % gave almost no increase in  $\Delta\text{CO}_2$ , whereas it significantly affected  $\Phi_e$ . Increased values of  $\Phi_e$  were observed in air with 21 % O<sub>2</sub>, though the variation range was considerably small compared to that in *V. radiata* (Fig. 1). Nevertheless, this fact may suggest that in this C<sub>4</sub> species a small amount of chemical energy is consumed through photorespiration.

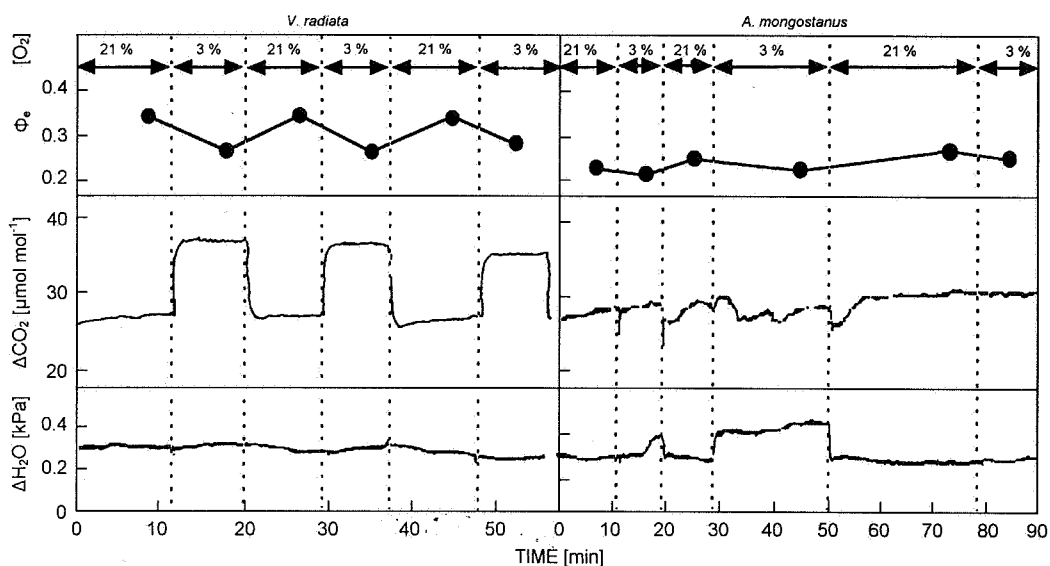


Fig. 1. Time courses of quantum yield of photosystem 2 ( $\Phi_e$ ), the difference of CO<sub>2</sub> concentration ( $\Delta\text{CO}_2$ ), and the difference of H<sub>2</sub>O vapour pressure ( $\Delta\text{H}_2\text{O}$ ) between sample air and reference air in *V. radiata* and *A. mongostanus* leaves with alternate changes between 21 and 3 % O<sub>2</sub> in the ambient air at 1 600  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD and leaf temperature of  $30.2 \pm 0.6$  °C.

Table 2. Values of parameters related to electron transport, photosynthesis, and photorespiration in *V. radiata* and *A. mongostanus* measured in air containing 3 and 21 % O<sub>2</sub>. For symbols see the list of abbreviations.

	<i>V. radiata</i> (C <sub>3</sub> )		<i>A. mongostanus</i> (C <sub>4</sub> )	
	21 % O <sub>2</sub>	3 % O <sub>2</sub>	21 % O <sub>2</sub>	3 % O <sub>2</sub>
$\Phi_e$	0.326±0.004	0.265±0.004	0.249±0.011	0.229±0.011
$k_c$	-	4.63±0.12	-	5.05±0.22
$k_N$	8.03±0.15	-	5.56±0.20	-
$J_T$ [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ]	243.8±3.1	198.2±2.7	181.5±7.9	167.5±8.8
$P_N$ [ $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ ]	28.3±0.6	40.2±0.7	31.6±0.3	32.0±0.9
$J_T/P_N$	8.61±0.19	4.93±0.15	5.74±0.21	5.23±0.23
$P_G$ [ $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ ]	30.3±0.5	42.8±0.6	32.7±0.3	33.1±0.5
$g_s$ [ $\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ ]	0.303±0.009	0.300±0.012	0.298±0.006	0.353±0.054
$V_c$ [ $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ ]	37.6±0.8	42.8±0.6	33.9±0.1	33.1±0.9
$V_o$ [ $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ ]	14.6±0.8	-	2.41±0.47	-
$R_{PE}$ [ $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ ]	7.3±0.4	-	1.2±0.2	-
$R_{PE}/P_N$ [%]	25.7±0.9	-	3.8±0.8	-
$R_{PO}$ [ $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ ]	11.9±0.2	-	0.43±1.00	-
$R_{PO}/P_N$ [%]	42.2±0.9	-	1.4±3.3	-

The value of  $k_c$  in *A. mongostanus* was  $5.05 \pm 0.22$  (Table 2), a little higher than a theoretically determined minimum value of 4.89 in C<sub>4</sub> plants (Table 1). The value

of  $5.05 \pm 0.22$  was slightly lower than the value of 5.20 determined by Krall and Edwards (1992) in maize in air at 80 Pa CO<sub>2</sub> and 3 % O<sub>2</sub> under a high irradiance of 1 800

$\mu\text{mol m}^{-2} \text{s}^{-1}$  PPFD. The value of  $k_N$  was  $5.56 \pm 0.20$ , which was higher than  $k_c$  ( $5.05 \pm 0.22$ ) in *A. mongostanus*. This is the evidence that chemical energy consumption increases with the increase in  $\text{O}_2$  concentration in air, and this may suggest the functioning of photorespiration in this species.

The photorespiration ratio,  $R_{\text{PE}}/P_{\text{N}_2 1\%}$ , was 3.8 % in *A. mongostanus* (Table 2). Based on rates of incorporation of  $^{18}\text{O}_2$  into glycolate, the photorespiration ratio in maize was 3 % in mature leaves and 11 % in young seedlings (deVeau and Burris 1989). Furthermore, the photorespiration ratio estimated from the model of  $\text{C}_4$  photosynthesis by Jenkins *et al.* (1989) was also similar to that shown here for *A. mongostanus*. According to Ueno (2001), RuBPCO functions not only in bundle sheath cells but also in ordinary epidermal cells, guard cells, and companion cells, whereas phosphoenolpyruvate carboxylase was not detected in ordinary epidermal cells and parts of guard cells in *Amaranthus edulis*. Hence photorespiration may be, though slightly, constantly functioning in this species.

In the  $\text{C}_4$  species *A. mongostanus*, Chl fluorescence is emitted in mix from chloroplasts included both in mesophyll cells and in bundle sheath cells. Chl fluorescence emission in  $\text{C}_4$  plants was already determined by Mayne *et al.* (1975) using isolated chloroplasts from different  $\text{C}_4$  subtypes. They found a functional Chl activity ratio of 27 : 73 for mesophyll : bundle sheath in NAD-ME subtypes such as *A. mongostanus*. More detailed studies were conducted on Hill reaction and PS2 potential in  $\text{C}_4$  species by Ku *et al.* (1974) and Edwards *et al.* (1976).

In  $\text{C}_4$  species, as mentioned above,  $\Phi_e$  calculation is based on the total value of Chl fluorescence emitted from the two different tissues. If  $\text{C}_4$  and  $\text{C}_3$  cycles work independently consuming chemical energies produced by mesophyll cells and bundle sheath cells, respectively, it may be difficult to determine the value of  $k_c$  in *A. mongostanus*. However, the  $k_c$  value may be determined from such a mixed value of Chl fluorescence in  $\text{C}_4$  plants, because the chemical energy produced in mesophyll cells and bundle sheath cells is available in common for the use in  $\text{C}_3$  cycles in bundle sheath cells through a chemical energy shuttle. Hatch (1987) and Leegood (1997) demonstrated the shuttle for chemical energy transport between mesophyll and bundle sheath. In this shuttle a part of phosphoglyceric acid transported from the bundle sheath to mesophyll was reduced by NADPH in mesophyll, and triose phosphate formed from phosphoglyceric acid was returned to the bundle sheath to maintain pools of  $\text{C}_3$  cycle intermediates in all three subtypes of  $\text{C}_4$  species.

We conclude that not only photorespiration rate in  $\text{C}_3$  species can be more correctly evaluated but also a low rate of photorespiration in  $\text{C}_4$  species can be detected based on the chemical energy production and consumption balance using the Chl fluorescence quenching diagnosis. The values obtained by this method are useful to deepen understanding of the functional role of photorespiration *in situ*, and the accumulation of data becomes valuable in elucidating the specific and variety features in growth sustainability and stress tolerance in relation to the dissipation of excessive energy in crops.

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