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Photorespiration Rate in Mungbean (*Vigna radiata* (L.) Wilczek) Leaves – A further estimation from the quantum yield at photosystem II–.

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Photorespiration rates (PR) in peeled (PL, without epidermis) and nonpeeled (NL) leaves of mungbean (*Vigna radiata* (L.) Wilczek) were estimated from electron transport situations at photosystem II using the chlorophyll fluorescence quenching diagnosis. The number (k_1) of electron equivalents required to produce 9 mol adenosine 5'–triphosphate (ATP) for fixing 1 mol CO_2 was experimentally determined 4.62. Also the number (k_2) of electrons required for releasing 1 mol CO_2 in photorespiration was estimated 7.44 or 9.49 on the assumption that 14.5 or 18.5 mol ATP was consumed for 1 mol CO_2 releasing, respectively. The simultaneous equations were constructed with the parameters of k_1, k_2 and related elements to calculate PR, and the mutual relationships in energy balance among PR, gross photosynthetic rate (Pg), total photosynthetic rate (TC, TC=PR+Pg) and PR ratio (PR/TC) were discussed. The value of PR, 1.75 or 2.05 μmol m^{-2} s^{-1}, in PL was estimated corresponding to k_2=7.44 or 9.49 in a light intensity of 300 μmol m^{-2} s^{-1} PPFD, and PR/TC was 18.2 or 20.6%, respectively. The estimation by chlorophyll fluorescence quenching diagnosis may give a more reasonable value of PR as compared with the PR obtained by the subtraction method (PR=Pg measured in 2% [O_2] air – Pg measured in 2% [O_2] air).

INTRODUCTION

The photorespiration is considered to have a negative effect on CO_2 intake or biomass production in crops, but this phenomenon is also known to play an important role in protecting the photosynthetic function in plants grown under the stress conditions such as drought and high light intensities. The photorespiration rate (PR) in a leaf is usually estimated by subtracting the photosynthetic rate (Pg) measured in the atmospheric air from that measured under the photorespiration–restricting air condition with a high [CO_2] and/or low [O_2]. The subtracted value are used for the estimation of photorespiratory characteristics in species and cultivars. However, the value of PR obtained in such conditions is predicted to be far different from the actual value in a leaf placed in the natural atmospheric air. In order to know the exact value of PR, another approach is necessary. We examined here the estimation of PR from the electron transport situation of photosystem II (PSII) using the chlorophyll fluorescence quenching diagnosis.

The situation of electron transport from PSII to the sites of CO_2 assimilation and photorespiration is diagnosed by the chlorophyll fluorescence quenching analysis described by Genty *et al.* (1989), Harbinson *et al.* (1990), Krall *et al.* (1990, 1991a, 1991b), Marco *et al.* (1990) and Schreiber *et al.* (1986, 1998). Tokuda *et al.* (1999)

already tried to calculate PR in mungbean leaves from the electron transport. In this trial, they assumed that 9 and 13.5 mol adenosine 5'–triphosphate (ATP) were consumed to fix and release 1 mol CO_2 in assimilation and photorespiration, respectively. However, the energy consumption in photorespiration may be differently estimated depending on how we define the photorespiratory category in relation to ATP consumption. In our study, based on the further estimation of the energy consumption in photorespiratory category, PR of mungbean leaf was calculated and the photorespiratory characteristics were discussed.

MATERIAL AND METHODS

Leaves of mungbean cultivar, Chinese, grown in fertilized 8–L pots were used as the experimental material. CO_2 exchange rate (CER) in detached leaves with and without epidermis was measured with an assimilation chamber (LD–2, Hansatech, UK) of open system (Tokuda *et al.*, 1999), and the sampled air was monitored with an infrared CO_2 analyzer (LI–6262, LI–COR, USA). A leaf, the abaxial epidermis of which was peeled, is termed here PL, and an nonpeeled leaf is NL. Two leaves, Leaf–A and Leaf–B, were used here; in the former the effect of epidermis peeling (change from NL to PL) on photosynthesis, photorespiration and quantum yield (Φe) at photosystem II (PSII) was examined, and in the latter the effect of $[O_2]$ variation from 20% and 2% on these parameter values in PL ($PL_{20\%}$ and $PL_{2\%}$) were investigated.

The chlorophyll fluorescence quenching was carried out simultaneously with the measurement of CER in Leaf–A and Leaf–B. The intensity of chlorophyll fluorescence at PSII was monitored with a portable fluorescence measurement system (MFMS–2T, Hansatech, UK). The initial fluorescence (F_o) of the leaf of unphotosynthetic situation was measured using a dark–adapted leaf under a measuring beam (3.2 μmol m^{-2} s^{-1} PPFD, 4.8 kHz) and then a 2 sec pulse illumination of 1,800 μmol m^{-2} s^{-1} PPFD was given to determine the maximum fluorescence (F_m). After this, the leaf was redarkened for 2 min, and then a red actinic light (300 μmol m^{-2} s^{-1} PPFD) was continuously illuminated. Fluorescence (F_s) and CER were simultaneously monitored and fluorescence peaks (F_m') were measured by giving pulses of white saturation light.

Based on measurements of fluorescence, Φe was calculated from the equations (1).

$$\Phi e=(F_m'-F_s)/F_m' \quad \cdots\cdots\cdots\cdots (1)$$

The equation (2) represents the relationship between gross photosynthetic rate (Pg) and Φe (Genty *et al.*, 1989).

$$Pg=\Phi e\times L\times 0.5\times i/k \quad \cdots\cdots\cdots\cdots (2)$$

where L is a light intensity supplied to a leaf; L=300 μmol m^{-2} s^{-1} PPFD is used here. i is the ratio of the photon absorbed by chlorophyll to the incident photon; i= 0.8 is used here.

Assuming that the photon is evenly distributed to the two photosystems, 0.5 is used in the equation (2). The parameter k is the number of electron equivalents required to reduce

1mol CO_2 and calculated from the equation (3).

$$k = \Phi e \times L_0 / Pg \quad \cdots\cdots\cdots\cdots (3)$$

where $L_0 = 0.5 \times L \times i$.

It may be considered that when photorespiration is performed in leaf, light energy supplied to PSII is shared into two functions of CO_2 assimilation and photorespiration. This relationship is expressed as the equations (4) and (5).

$$L_0 = L_1 + L_2 \quad \cdots\cdots\cdots\cdots (4)$$

$$Pg = \Phi e \times L_1 / k_1 - \Phi e \times L_2 / k_2 \quad \cdots\cdots\cdots\cdots (5)$$

where L_1 and L_2 are light energy shared from L_0 and used for CO_2 fixation and photorespiration, respectively. Similarly, k_1 and k_2 are the number of electrons required for CO_2 assimilation and photorespiration, respectively. The values of $\Phi e \times L_1 / k_1$ and $\Phi e \times L_2 / k_2$ indicate the total CO_2 assimilation rate (TC) and PR, respectively.

RESULTS AND DISCUSSION

Table 1 shows the parameter values of Pg, Φe, L_0 and k determined in Leaf-A and Leaf-B under the different $[CO_2]$ and $[O_2]$ conditions at leaf temperatures of 35°C and 25°C. As shown here, the difference in Pg between the values in PL and NL represents the effect of epidermis peeling. It may be considered that the stomatal resistance removal by epidermis peeling increases CO_2 intake rate and simultaneously enhances intercellular $[CO_2]$, with a result of the restriction of photorespiration.

Table 1. The values of Pg, Φe and k measured in different $[O_2]$ and leaf temperatures.

		Measurement condition				Obtained values		
		$[CO_2]$ μmol mol^{-1}	$[O_2]$ %	Temp.	L_0 μmol m^{-2} s^{-1}	Pg μmol m^{-2} s^{-1}	Φe	k
Leaf-A	NL	350	20	35	120	4.39	0.487	13.31
	PL	350	20	35	120	9.65	0.419	5.21
Leaf-B	$PL_{20\%}$	350	20	25	120	7.88	0.509	7.75
	$PL_{2\%}$	350	2	25	120	12.50	0.481	4.62

Leaf-A (NL and PL) was used for the epidermis peeling test, and Leaf-B ($PL_{20\%}$ and $PL_{2\%}$) was measured in 20% and 2% $[O_2]$ air. $L_0 = L \times 0.5 \times i$; 120 μmol m^{-2} s^{-1}=300 μmol m^{-2} s^{-1} $\times 0.5 \times 0.8$. The values of k were calculated from the equation (3).

As shown in Leaf-B, Pg of 7.88 μmol m^{-2} s^{-1} measured in 20% [O_2] increased to 12.50 μmol m^{-2} s^{-1} in 2% [O_2]. The value of k obtained under the photorespirationless condition (2% [O_2] air) was 4.62, which seems to be close to the theoretical value (k=4).

The determination of k_1 and k_2 values is necessary for the estimation of PR from the equations (4) and (5). Tokuda *et al.* used k_1=4.62 and k_2=6.93 which were determined on the assumption that 9 mole ATP was required to assimilate 1 mol CO_2 and 13.5 mol ATP was required to release 1 mol CO_2 in photorespiration. But here we have assumed two cases where 14.5 (the case I) or 18.5 mol (the case II) ATP is needed to release 1 mole CO_2 in photorespiration. When 9 mole ATP is required for assimilation and 14.5 mol ATP is for photorespiration, the ratio of k_1 : k_2 is assumed to be 9:14.5. Based on this ratio, k_1 and k_2 are calculated 4.62 and 7.44, respectively (the case I in Table 2). In the case II, where the nitrogen metabolic reaction is included in photorespiration category, ATP consumption per 1 mol CO_2 releasing is estimated 18.5; accordingly the ratio of k_1 : k_2 is 9:18.5 and thus the values of k_1 and k_2 are 4.62 and 9.49, respectively. By substituting k_1=4.62, k_2=7.44 or 9.49 and the measured values of Pg, L_0 and Φe into the equations (4) and (5), the values of TC, PR, L_1 and L_2 are calculated .

Table 2. The estimated number, k_1 and k_2, of electron equivalents required for 1 mol CO_2 fixing in assimilation and releasing in photorespiration, respectively.

Case	I	II
k_1	4.62 (9)	4.62 (9)
k_2	7.44 (14.5)	9.49 (18.5)

The numbers between parentheses are ATP mol required in assimilation or photorespiration (fixing or releasing of 1 mol CO_2, respectively).

TC, PR, photorespiration ratio (PR/TC), L_1 and L_2 are listed in Table 3. The values of TC, PR and PR/TC varied with k_2, but the difference was not large in each parameter between the cases I and II. For example, the values of PR/TC in NL of Leaf-A were 41.8 and 37.9% in the case I and II, respectively; their difference was about 10%. The PR/TC calculated by Tokuda *et al.* (1999) based on k_1=4.62 and k_2=6.93 using the same leaf under the same condition was 42.9%, showing not so large difference from the above values.

So far PR was usually calculated by subtracting (PR=Pg measured in 2% [O_2] – Pg measured in 20% [O_2]). In Table 4, PR in Leaf-B calculated by the subtraction method is shown together with the related parameters to compare with the PR estimated from electron transport using the equations (4) and (5) in the case II (k_1=4.62 and k_2=9.49). The value of PR obtained by the subtraction method (PL_{sub}) was 4.62 (12.50-7.88=4.62) μmol m^{-2} s^{-1}, which was significantly larger than that (PR=1.75 μmol m^{-2} s^{-1}) estimated from electron transport. If CO_2 assimilation (TC=12.50 μmol m^{-2} s^{-1}) and photorespiration (PR=4.62 μmol m^{-2} s^{-1}) are performed in a light energy of 120 μmol m^{-2} s^{-1} PPFD supplied to PSII, the recalculated value of Φe comes up to 0.846, but the actually measured Φe is

Table 3. Predicted values of TC, PR, PR/TC, L_1 and L_2 in the cases of I and II.

		Pg	Φe	TC	PR	PR/TC	L_1	L_2	L_o
		μmol m^{-2} s^{-1}		μmol m^{-2} s^{-1}		%	μmol m^{-2} s^{-1}		
Case I	Leaf-A								
	NL	4.39	0.487	7.55	3.16	41.8	71.7	48.3	120
	PL	9.65	0.419	10.12	0.47	4.7	111.7	8.4	120
	Leaf-B								
	$PL_{20\%}$	7.88	0.509	9.93	2.05	20.6	90.1	29.9	120
	$PL_{2\%}$	12.50	0.481	12.50	0	0	120	0	120
Case II	Leaf-A								
	NL	4.39	0.487	7.10	2.71	37.9	67.4	52.6	120
	PL	9.65	0.419	10.05	0.40	4.0	110.9	9.1	120
	Leaf-B								
	$PL_{20\%}$	7.88	0.509	9.63	1.75	18.2	87.4	32.6	120
	$PL_{2\%}$	12.50	0.481	2.50	0	0	120	0	120

In the case I, k_1=4.62 and k_2=7.44 were used in calculation, and in the case II k_1=4.62 and k_2=9.49 were used. See Table 1 for the case I and II. The values of TC, PR, L_1 and L_2 were calculated from the equations (4) and (5) by substituting the values of Pg, Φe, k_1, k_2 and L_o.

Table 4. The parameter values estimated in the case II and calculated by subtraction method.

	Pg	TC	PR	PR/TC	Φe	$L_1 \times$ Φe	$L_2 \times$ Φe	$L_o \times$ Φe
	μmol m^{-2} s^{-1}			%		μmol m^{-2} s^{-1}		
Leaf-B in the case II								
$PL_{20\%}$	7.88	9.63	1.75	18.2	0.509	44.5	16.6	61.6
$PL_{2\%}$	12.50	12.50	0	0	0.481	57.7	0	57.7
PL_{sub}	7.88	12.50	4.62[a]	37.0	0.846[e]	57.7[b]	43.8[c]	101.5[d]

PR[a]=4.62 μmol m^{-2} s^{-1} was determined by PR=Pg of $PL_{2\%}$–Pg of $PL_{20\%}$. 57.7[b] and 43.8[c] were calculated as TC$\times k_1$ and PR$\times k_2$, respectively; k_1=4.62 and k_2=9.49 were used here. 101.5[d] was the sum of 57.7[b]+43.8[c]. 0.846[e] was calculated by 101.5 μmol m^{-2} s^{-1} /120 μmol m^{-2} s^{-1}.

0.509 here. There is a large difference between both values of Φe, which may suggest that PR=4.62 μmol m^{-2} s^{-1} is a considerably overestimated value.

The photorespiration has been regarded as having a functional role of a safety valve for protecting photosynthetic system under the stress conditions. Therefore it may be predicted that a leaf with high PR/TC is superior in keeping its photosynthetic activity longer. The genetic variation in photorespiratory characteristics may become an important selection point in breeding of plant with longer-lived leaves. For making possible such a selection it is indispensable to know the exact value of PR in leaf. From

the result obtained in our study it may be concluded that more reasonable values of PR and PR/TC are predicted based on the electron transport situation diagnosed by the chlorophyll fluorescence quenching analysis.

The Mehler reaction is known as another function of consuming the chemical energy produced by photosystem. The energy consumption in this reaction is differently estimated with a wide range from a small amount (Padmasree and Raghavendra, 1998) to such a large amount as reaching several tens percent of the total chemical energy produced in photosystem (Osmond and Grace, 1995). Environmental conditions are known to be greatly effective on the Mehler reaction, but we predict that the energy consumption in the Mehler reaction is not so large in many cases. Particularly, in our experiment, the light intensity (300 μmol m^{-2} s^{-1} PPFD) used was below 50% of the photosynthetically saturating light, under the condition of which the Mehler reaction seems to be almost negligible in chemical energy consumption.

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