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Variation of Alternative and Cytochrome Respiration during Ripening in Rice Leaves

Kwang-hong Lee*†

Graduate School of Agriculture and Life Sciences, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
**Photosynthesis Research Labotory, National Institute of Agrobiological Sciences, 2-1-2 Kannondai, Tsukuba, Ibaraki 305-8602, Japan*

ABSTRACT: The variation of alternative and cytochrome respiration during ripening in rice leaves (*Oryza sativa* L. cv. Takanari) was examined. The capacity of both respiration pathway was measured by inhibitor titration method using gas-phase oxygen electrodes. The alternative respiration rate decreased from 31.3% of the total respiration rate at around heading to 11.7% at 34 days after heading in the first fully expanded leaf from the top, and further to 6.4% at 34 days after heading in the fourth leaf from the top. In contrast, the proportion of cytochrome respiration to total respiration increased with leaf senescence. The possible cause of alternative respiration as either an increase in inefficient respiration or a decrease in growth efficiency during ripening was discussed.

Keywords **:** alternative respiration, cyanide resistant, cytochrome, growth efficiency, rice, senescence.

Plants have two electron transport pathways for dark respiration: the cytochrome pathway and the alternative pathway (Siedow and Umbach, 1995). The alternative pathway is important for physiological adaptability against environmental stresses (Wagner and Moore, 1997). The alternative pathway takes electrons directly from the ubiquinone pool. No ATP is produced; free energy is used to reduce oxygen to water and then is given off as heat. When NADH is used for electron transport, the efficiency of carbon used to produce ATP is less than 1/3 of that in the cytochrome pathway (Lambers, 1982). For this reason, the alternative pathway has been thought to reduce plant productivity (de Visser and Lambers, 1983). A pea hybrid line that was thought to lack the alternative pathway accumulated greater dry weight (Musgrave *et al.*, 1986), but this line was later shown to possess the pathway (Goyal *et al.*, 1991). Thus, information about the alternative pathwayís contribution to plant productivity is quite limited.

During ripening, the respiration rate of rice leaves makes up a large proportion of whole-plant respiration rate, while its growth efficiency decreases (Yamaguchi *et al.*, 1975). However, there is no idea about their relations with alternative respiration. Here, we examined the variation of alternative and cytochrome respiration, and discussed whether decline of growth efficiency is caused by the alternative respiration pathway in rice leaves during ripening.

†Corresponding author: (Phone) +81-298-38-7074 (E-mail) khlee@affrc.go.jp
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MATERIALS AND METHODS

Rice cv. Takanari was hydroponically grown as described by Lee and Akita (2000) in a culture solution of 10 mg L^{-1} nitrogen. The nitrogen concentration was initially 5 mg L^{-1}, and doubled in the 35 days from transplanting to harvest.

Alternative respiration was measured polarographically with gas-phase Clark-type oxygen electrodes (LD2 and LD2/2) (Hansatech Co., UK). The temperature of the water circulating around the chamber was 25.0°C, and room temperature was maintained at 24°C. Buffer solution (pH 7.2) contained 50 mM HEPES and 0.5 mM $CaSO_4$, with 0.1% Tween-20 and 1% ethanol added to improve the affinity of materials. Tween-20 and ethanol did not affect the respiration rate. Alternative respiration was measured using inhibitor titration. KCN dissolved in distilled water was used as the cytochrome pathway inhibitor; n-propyl gallate dissolved in 2-methoxy ethanol was used as the alternative pathway inhibitor.

The total respiration rate (V_t) consists of the cytochrome (V_c), alternative (V_a), and residual (V_r) respiration rates:

$$V_t = V_c + V_a + V_r \quad (1)$$

The total respiration rate is the respiration rate without inhibition, and the residual respiration rate is the rate without inhibition by cytochrome or alternative pathway inhibitors.

The middle part of the first leaf from the top was cut transversely into 4 to 6 slices, each 2.5 cm long. The slices were put in the buffer solution, subjected to a weak vacuum for about 1 minute in order to increase the infiltration of inhibitors, and incubated for about 20 minutes in the dark at room

temperature; then V_t was measured. KCN was added to the buffer solution to measure respiration without the cytochrome pathway (V_a+V_r), and both propyl gallate and KCN were added to the buffer solution to measure residual respiration (V_r). Slices were subjected to a vacuum in each of these inhibitor solutions and incubated for 1 hour in the dark; then the respiration rates were measured for about 30 minutes at each step. The measurement of all respiration rates took approximately 2.5 hours. Leaf slices were dried and measured dry weight. The cytochrome respiration rate was calculated as $V_t-(V_a+V_r)$, and the alternative respiration rate was calculated as $(V_a+V_r)-V_r$. The rates were expressed as the rate of oxygen uptake per unit dry weight per minute ($\mu g\ g^{-1}min^{-1}$).

Growth efficiency was calculated using the following equation (2) (Tanaka and Yamaguchi, 1968).

$$\text{Growth efficiency} = CGR/P_g \quad (2)$$

where CGR is crop growth rate ($g\ m^{-2}d^{-1}$), and P_g is gross photosynthesis ($g\ m^{-2}d^{-1}$). P_g is the sum of the rates of crop growth and dark respiration.

The whole-plant dark respiration rate was measured by infrared CO_2 analyzer, as explained in Lee and Akita (2000). A coefficient (30/44) was used to convert dark respiration rates from CO_2 to CH_2O. The dark respiration rate ($g\ m^{-2}d^{-1}$) was calculated from the average of individual dark respiration rates at 25°C and the average daily temperature between two sampling periods, assuming a temperature coefficient of 1.8 (Lee and Akita, 2000). To measure plant dry weight, 6 to 12 plants were sampled and dried for at least 3 days at 80°C. The dry weight of dead organs was excluded from the dark respiration rate calculations. The crop growth rate ($g\ m^{-2}d^{-1}$) was calculated from the average rate of dry weight increase (including dead organs) between two sampling periods.

RESULTS AND DISCUSSION

The total respiration rate in the first fully expanded leaf from the top decreased during ripening (Fig. 1), from 9.82 $\mu g\ g^{-1}min^{-1}$ at the heading stage, to 8.72 $\mu g\ g^{-1}min^{-1}$ at 14days after heading (DAH) and 8.11 $\mu g\ g^{-1}min^{-1}$ at 34 DAH. The respective alternative, cytochrome, and residual respiration rates were 3.07, 3.87, and 2.87 $\mu g\ g^{-1}min^{-1}$ at heading; 2.82, 3.54, and 2.35 $\mu g\ g^{-1}min^{-1}$ at 14 DAH; and 0.95, 4.80, and 2.35 $\mu g\ g^{-1}min^{-1}$ at 34 DAH. The V_r was almost constant during ripening, ranging from 27.0 to 29.2% of V_t. The ratios of V_a to V_t and V_c to V_t changed little from heading to 14 DAH, but varied from 14 to 34 DAH. The V_a ranged from 11.7 to 31.3% of V_t at heading and 34 DAH, respectively. The V_c ranged from 39.5 to 59.3% of V_t at heading and 34 DAH, respectively. Thus, the alternative respiration decreased while the cytochrome respiration increased during ripening.

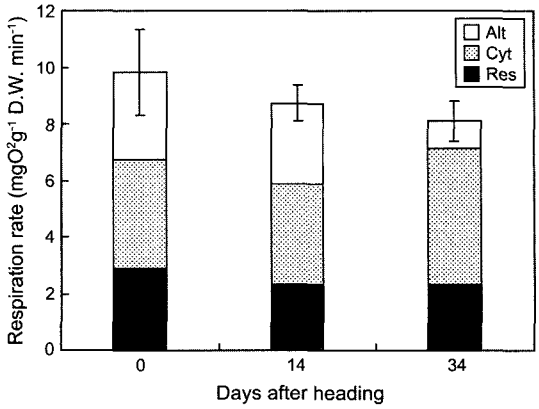

Fig. 1. Variation in the alternative (Alt), cytochrome (Cyt), and residual (Res) respiration rates in rice leaves. The standard deviation of total respiration rate is shown on the graph. Each measurement was replicated three times.

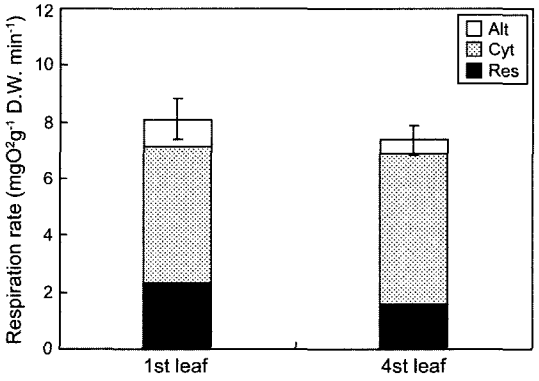

Fig. 2. Comparison of the alternative (Alt), cytochrome (Cyt), and residual (Res) respiration between the first and fourth leaf from the top of rice plants at 34 days after heading. The data for the first leaf is taken from Fig. 1. The standard deviation of each respiration rate is shown on the graph. Each measurement was replicated three times.

The effect of leaf senescence on respiration rate was further examined by comparing the first and the fourth leaf from the top at 34 DAH (Fig. 2). The fourth leaf was much more yellowish than the first leaf. The total, alternative, cytochrome, and residual respiration rates of the fourth leaf were 7.38, 0.47, 5.30, and 1.61 $\mu g\ g^{-1}min^{-1}$, respectively. The V_a further decreased from 11.7% of V_t in the first leaf to 6.4% in the fourth. The V_c increased from 59.3% of V_t in the first leaf to 71.8% in the fourth. The V_r slightly decreased from 29.0% of V_t in the first leaf to 21.8% in the fourth. Thus, the rate of alternative respiration and its ratio to V_t gradually decreased with senescence in rice leaves during ripening.

The effect of senescence on alternative respiration is controversial. Alternative respiration decreased with increasing

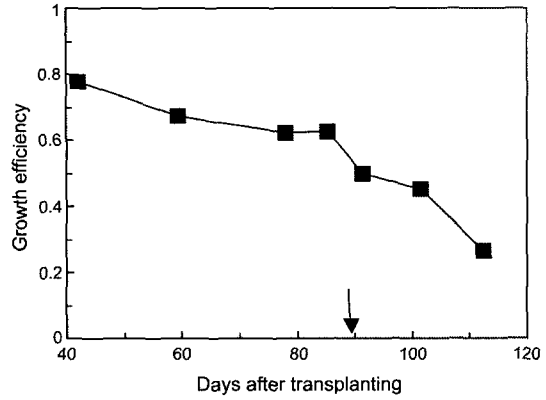

Fig. 3. Variation in growth efficiency during development in rice, cv. Takanari. Arrow indicates the heading date.

age in bean leaves (Azcon-Bieto *et al.*, 1983c), in sweet potato slices (Theologis and Laties, 1978) and in *Plantago* roots (Lambers *et al.*, 1981), but increased during the senescence of sugar beet leaves (Shugaev *et al.*, 1998).

Alternative respiration rates are increased by prolonged photosynthesis (Azcon-Bieto *et al.*, 1983b; Atkin *et al.*, 1993) and are also higher in leaves in light compared to at the end of the night (Azcon-Bieto et al., 1983a). These trends are related to the amount of soluble sugars (Azcon-Bieto *et al.*, 1983a, b, c). In rice, leaf photosynthesis decreases as leaves senesce after heading (Ishizuka, 1971), and the amount of soluble sugars in leaves decreases during ripening (Yoshida and Ahn, 1968). Thus, the soluble sugar content can also explain our results.

The contribution of dark respiration to plant productivity can be evaluated by growth efficiency (Tanaka and Yamaguchi, 1968). Growth efficiency sharply decreased during ripening (Fig. 3). It had a value of about 0.8 in the early growth stage and gradually decreased to about 0.6 during the vegetative growth period. After heading, it decreased sharply to less than 0.3. These data are consistent with an earlier report (Tanaka and Yamaguchi, 1968). Therefore, to increase crop productivity, growth efficiency should be increased, especially during reproductive growth periods. The decrease of growth efficiency during ripening could be related to the increasing proportion of dark respiration to gross photosynthesis. This proportion may increase due to an increase in respiration inefficiency, as well as an increase in maintenance respiration during ripening. Maintenance respiration rate increases with plant biomass (McCree, 1974). However, the mechanisms of inefficient respiration are not well known. If the proportion of the alternative to total respiration rate increases during ripening, the alternative pathway could explain respiration inefficiency, and the decrease in growth efficiency during ripening could be partially attributed to an increase in alternative respiration. However, our results suggest that the alternative respiration pathway is not responsible for the increase of inefficient respiration during ripening, or for the decrease of growth efficiency.

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