The Difference of Diurnal Changes in Photosynthesis in Rice Plants with Different Root Activities Induced by Soluble Starch Application to the Soil*

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Abstract: In order to verify the difference in diurnal changes in photosynthesis in rice plants with different root activities, the experiment was conducted with a control plant and a soluble starch-applied plant more than 16 days after soluble starch application. There was a larger difference in the diurnal changes in the photosynthesis and stomatal conductance, due to increase in Rubisco contents resulted from the application of soluble starch and nitrogen. Photosynthesis and stomatal conductance in the low-root activity plant were higher before 10 o'clock and lower at midday and in the afternoon. The response curves of the photosynthesis to photosynthetic photon flux (PPF) expressed a significant saturable curve and were obviously different between morning and afternoon under PPF over 1000 μmolm⁻²s⁻¹. An obvious diurnal change in the initial activity and activation rate of Rubisco carboxylation had been found. They all were higher at midday than in the early morning and late afternoon. The initial activity based on leaf area was significantly higher in the low-root activity rice plant, but its activation rate was obviously lower. Therefore, the difference in diurnal changes in photosynthesis between the different root activity plants can not be attributed to the difference in Rubisco activity, but mainly to the difference of the midday drop in stomatal conductance, which was significantly correlatable to photosynthesis.

Key words: Diurnal change, Photosynthesis, Rice plant, Root activity, Rubisco activation %, Rubisco content, Rubisco initial activity, Stomatal conductance.

根の生理的活性の異なる水稲稈葉の光合成速度の日変化の相違とその要因について：蔵 勯安・平沢 正・石原 邦（東京農工大学農学部）

要旨：根の生理的活性の異なる稈葉の光合成速度の日変化の相違とその要因を明らかにするため、potに生育させた第10葉抽出期および出穂開始期の稈葉を用いて、前報と同様に土壌中に可溶性デンプンと硫安を施し、根の生理的活性を低下させる処理を行った（処理稈葉）。処理稈葉では処理を行わなかった対照稈葉に比べて、a-アミラーゼ活性が小さかったが、蒸散の高い早朝には稈葉の光合成速度はやや高かった。一方、午前のおおむねと午後の処理稈葉の光合成速度、拡散伝導度はともに対照稈葉に比べて小さかった。この日変化を光強度と光合成速度との関係に書き直してみると、光一光合成曲線は飽和型を示し、光強度1000 μmolm⁻²s⁻¹以上では、対照稈葉では午前と午後の光合成速度にほとんど相違はみられないが、根の生理的活性の低い処理稈葉では同じ光強度で午後の光合成速度は午前に比べ明らかに低かっ

There are some reports\textsuperscript{7,18} on the diurnal change in photosynthesis and effect of potassium nutrition on it in rice grown in the field. There are a few publications, however, to have shown the difference in this change of the plant with various physiological activities of root. In our previous paper\textsuperscript{9}, we reported that the photosynthesis, stomatal conductance and leaf bleeding rate in the soluble starch-applied rice plant decreased along with a declination of root activity during 2–9 days after soluble starch treatment, while the initial activity of Rubisco carboxylation and Rubisco content were not much changed. The lower stomatal
conductance may be a dominant factor for low photosynthesis in the treated plant following soluble starch application, but in 10 days or more after this kind of treatment, when the root activity in treated plant still remained lower, the photosynthesis measured in the morning recovered or were even higher than the control plants. It is still not understood whether this recovery of photosynthesis in the low root activity plant can remain in a whole day. In previous paper we hypothesized that a severe midday depression in photosynthesis in low root activity plants would occur at low atmospherice relative humidity and higher leaf temperature in the midday due to a large amount of water loss by transpiration. In addition, we do still not find available reports on the relationship of the diurnal changes in photosynthesis with the Rubisco carboxylation activity in rice plant although it had been reported in other plant\(^9\). To verify the hypothesis, to further illuminate the effect of low root activity on photosynthesis in leaf, and to discover whether diurnal change in photosynthesis involves into the Rubisco activity, we measured the diurnal change in photosynthesis, stomatal conductance, initial and total activities of Rubisco carboxylation, Rubisco content and water contents in the leaves of the plants with different root activities.

**Materials and Methods**

1. **Plant material**
   The rice (*Oryza sativa* L. cv. Nipponbare) was planted in the pot and managed as described in previous paper\(^8\).

2. **Soluble starch application to soil**
   The treatment was carried out at 10th leaf stage on June 25 and at the initial heading stage on August 20 as described in previous paper\(^8\).

3. **The measurement of root activity**
   Root activity measurement was referred to \(\alpha\)-naphthylamine oxidation method\(^8\).

4. **The measurement of photosynthetic photon flux, photosynthesis and stomatal conductance**
   All of the above in the fully expanded top leaf or flag leaf attaching to the main stem were measured with LI-6200 (LI-COR) portable photosynthetic and transpiration system under the outside conditions where the plant grew. The data were an average of 4 leaves.

5. **The assay for initial and total activities of Rubisco carboxylation**
   The assay were conducted following the methods as described by Lilley et al.\(^{10}\) and our previous paper\(^8\). The measurement for the total activity began immediately after RuBP was injected the cell with the enzyme activated in a medium containing 33 mM Tris–HCl pH7.5, 0.67 mM EDTA, 33 mM MgCl\(_2\) and 10 mM NaHCO\(_3\) at 30°C for 10 min and the same medium as used for the initial activity\(^8\).

6. **The determination of Rubisco contents**
   The quantity of Rubisco was done with the single radial immunodiffusion method illustrated by Koide et al.\(^9\) and Sugiyama et al.\(^21\) using Rubisco purified from rice seedling according to Makino et al.\(^11\) as a standard.

7. **Determination of water content**
   The other except the part for using Rubisco measurement was rapidly sealed into a cuvette and weighed and then dried in oven at 90°C to stable weight.

**Results**

1. **The photosynthesis and root activity**
   As illustrated in Table 1, the root activity was significantly lower in the plant with soluble starch application than in the control plant at both treatment stages, but in the morning photosynthesis in the treated plant was higher in the control plant as well as described in our previous paper\(^8\) because the former had much more Rubisco content resulted from the adding nitrogen applied with soluble starch at same time.

2. **The diurnal change of photosynthesis**
   Before 10 o'clock, the photosynthesis of the fully expanded top leaf, the 13th leaf, was higher in the 15 g soluble starch-treated plant than in control plant on the 36th day after soluble starch was applied at the 10th leaf stage. The same photosynthesis took place in both plants at about 10:30, and then the photosynthesis declined in the soluble starch-treated plant and the difference between the control and the treated plant became increasingly greater (Fig. 1A) until a shower (an arrow in Fig. 1). When applied at the initial heading stage, soluble starch did not result in a fall in photosynthesis on flag leaf before 10 o'clock (Fig. 2A) either, but resulted in an obvious fall in the photosynthesis from 11:00
Table 1. The effect of application of soluble starch with nitrogen to the soil on the root activity ($\alpha$-naphthylamine oxidation), photosynthesis and Rubisco content.

<table>
<thead>
<tr>
<th>Date</th>
<th>Treatment</th>
<th>Day after treatment</th>
<th>Root activity ($\mu$g g$^{-1}$FW)</th>
<th>Photosynthesis ($\mu$mol CO$_2$ m$^{-2}$s$^{-1}$)</th>
<th>Rubisco content (g m$^{-2}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 31</td>
<td>Control</td>
<td>36</td>
<td>59.9±2.7</td>
<td>31.97±2.30</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>SA</td>
<td></td>
<td>44.2±2.0</td>
<td>34.24±1.90</td>
<td>—</td>
</tr>
<tr>
<td>Sept 5</td>
<td>Control</td>
<td>16</td>
<td>32.7±9.4</td>
<td>24.81±4.51</td>
<td>4.364±0.077</td>
</tr>
<tr>
<td></td>
<td>SA</td>
<td></td>
<td>31.8±3.7</td>
<td>28.31±4.44</td>
<td>5.831±0.296</td>
</tr>
</tbody>
</table>

Control and SA indicate separately without and with 15 g soluble starch adding 2.5 g ammonium sulfate to the soil per pot.

Data show average ± standard error of 4 plants with 3 measurements, respectively. Photosynthesis measurement was conducted at 8:00~10:00 am.

![Graph showing the diurnal changes in photosynthesis and PPF](image)

**Fig. 1.** The diurnal changes in the photosynthesis (A) and the response curves (B) of photosynthesis to photosynthetic photon flux (PPF) in rice plants with different root activities on July 31. The black and white circles or squares represent the control and low root activity plants, respectively. Square symbols stand for the date before the depression in the morning. An arrow shows a shower.

to 14:00.

It is impossible to control the photosynthetic photon flux (PPF) at exactly the same levels between the control and the treated plants outside in both days with a little frequent cloud, so we plotted the response curves of photosynthesis to PPF to reflect the difference between both plants more clearly. Fig. 1B and 2B pronounced that the photosynthesis were much lower in the low-root activity plant caused by soluble starch application than in the control plant under PPF over approximate 1000 $\mu$mol m$^{-2}$s$^{-1}$. The difference in photosynthesis between in the morning (square symbols) and in the midday (circle symbols) got larger in the treated plant while slight difference in the control plant appeared.

3. The diurnal change of stomatal conductance and leaf water content

The diurnal change of stomatal conductance shown in Fig. 3 expressed it was largest in the early morning and thereafter rapidly dropped with an increase in PPF, and rose slightly again at about 11 o'clock and decreased again in the afternoon in both days, except for a shower on July 31. Compared to the control plant, the low-root activity plant possessed higher stomatal conductance in the morning and less at noon and in the afternoon, as reported by as well as that in the nitrogen-applied plant because high nitrogen caused a poor-developed root system and lessened the conductance in the afternoon. Under the high sunlight intensity in the midday, photosynthesis was significantly correlative to
stomatal conductance (the part in the broken line square), implying that the lower stomatal conductance is a mainly limited factor responsible for the lower photosynthesis in the low-root activity rice plant (Fig. 4).

The leaf water content on basis of dry weight was significantly decreased at midday as compared to that in the morning and evening but no difference between the control and low-root activity plants was observed until 14:00 (Fig. 5D). The lower water content in low-root activity plant after 14:00 demonstrated a low capacity of active absorption of water in root.

4. The diurnal change of activity of Rubisco carboxylation

An obvious diurnal change in initial activity, specific initial activity and activation rate of Rubisco carboxylation showed in Fig. 5. They all were higher at midday than in the early morning and late afternoon. The initial activity based on the leaf area was significantly higher in the low root activity plant (the white circles in Fig. 5A) treated with soluble starch plus ammonium sulfate than in the control plant (the black circles in Fig. 5A), but the activation rate was obviously lower in the former (the white circles in Fig. 5B) while
Fig. 4. The relationship between stomatal conductance and photosynthesis under high light intensity during the depression of photosynthesis in the midday on July 31 (A) and September 5 (B) in 1993. The black and white circles represent the control and low root activity plants, respectively. Data are from the parts in the broken–line squares in Fig. 3.

Fig. 5. The diurnal changes of initial activity (A), activation % (B) and specific activity (C) of Rubisco carboxylation, and leaf water content (D) on September 5. The black and white circles represent the control and low root activity plants, respectively.

the specific activity on the basis of Rubisco content was similar in the both plants (Fig. 5C).

Discussion
As shown in Table 1, the photosynthesis from 8:00 to 10:00 was not lower but higher in the low–root activity plant induced by treatment with soluble starch plus nitrogen. The probable reason for this is an increase in initial Rubisco carboxylation activity of the determined leaf introduced by additional nitrogen. There are many reports in which nitrogen promoted photosynthesis and simultaneously increased in Rubisco activity or content,

12,13,14), and our results also displayed an increase in initial Rubisco activity in the treated plant (Fig. 5A). These results suggest that a proper application of nitrogen to rice plant grown in the high-reduced paddy is beneficial to avoid leaf senescence and maintain photosynthesis, at least in the early morning, and
showed a practical significance for high-yielding culture of rice in the fields where strong reduction was often caused by flooding or using fresh organic manure because our complementary experiment in which soluble starch was applied without additional nitrogen showed lower photosynthesis even in the early morning (data not shown).

When it had been recovered in the early morning, the photosynthesis in low-root activity plant was still lower than that in the control plant at midday, and the treated plant appeared lower light saturation (Fig. 1 and 2). This kind of difference in diurnal change of photosynthesis between the control and treated plant was very similar to that of stomatal conductance (Fig. 3). The fact that there was a significant correlation between the stomatal conductance and the photosynthesis during the midday depression (Fig. 4) pronounced our previous hypothesis that lower stomatal conductance in low root activity plant accelerated the midday depression in photosynthesis upon lower air relative humidity and higher temperature.

As the midday depression of stomatal conductance in the low-root activity plant was not caused by an exceeded loss of leaf water (Fig. 5D), the question is how the stomata receive the signal of low root activity. It is well known that potassium adjusts the stomata open and close, and there are some early reports on the respiratory inhibitors obstructing potassium absorption of root, so it is possible that low-root activity rice have lower leaf potassium content which results in the midday closure of stomata -- unlike that our previous report in which we deduced that the possibility can be excluded in a short time after soluble starch treatments. Recently, there have been reports of the lower cytokinin content in low root activity rice cultivar and on a high ABA content as a signal closing stomata as root system encountered soil drying. As reported that the soluble starch treatment caused a depression of water potential of leaf in the treated rice plant in the middle of day, our result showed that leaf water potential were -0.14, -0.12, -0.45 and -0.12 MPa in the leaf of control plants, and -0.14, -0.12, -0.58 and -0.26 MPa in the leaves of the low-root activity plants at 6, 9, 12 and 15 o'clock on September 11, respectively, indicating the leaf water potential in the afternoon was lower in the low root activity plant than in the control plant. It is possible that the leaf water potential rather than leaf water content responses for stomatal closure at midday.

From Fig. 5A, we make sure that a severer midday depression in photosynthesis in the low root activity plant is not attributed to Rubisco activity. It is very interested in that there were the obvious diurnal changes in the initial activity (Fig. 5A), activation rate (Fig. 5B) and specific activity (Fig. 5C) of Rubisco carboxylation and all these were higher in the midday than in the early morning and late afternoon. The specific initial activity of Rubisco carboxylation, which rules out the error of initial activity derived from the different leaves containing different Rubisco content, showed an obvious diurnal change. It did not agree with a putative pattern in which the activity is considered to be unchanged during a day except for early morning, although the similar result appeared in soybeans. In chloroplast some metabolites binding to the active site of Rubisco can result in the slow catalytic formation of Rubisco, which means that some ways must exist in vivo to restore and maintain the Rubisco carboxylation activity. It is known that the low catalytic status can be activated in vivo by Rubisco activase through hydrolyzing ATP. These results might suggest that to avoid wasting ATP in the early morning and in the evening upon not enough irradiance, only a part of Rubisco is activated when its total activity exceeds the need of CO₂ assimilation. Maybe, in the early morning and in the evening, there is another state of Rubisco which can not be activated in vitro by CO₂ and Mg²⁺ besides the activated state and the inactivated state which can be activated in vitro by CO₂ and Mg²⁺ in suitable pH. This merits further exploration using more samples and days so as to collect enough evidence.

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References

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