

INFLUENCE OF GROWTH TEMPERATURE AND  
NITROGEN NUTRITION ON PHOTOSYNTHESIS  
AND NITROGEN METABOLISM IN THE RICE  
PLANT (*ORYZA SATIVA* L.)<sup>1,2</sup>

YUH-JANG SHIEH and WEN-YUAN LIAO

*Institute of Botany, Academia Sinica  
Nankang, Taipei, Taiwan 11529, Republic of China*

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**Abstract**

The four-leaf-stage seedlings of two rice varieties were grown in the combinations of 5 levels of nitrogen (N) (from 5 to 80 ppm N) and three temperature regimes (day/night temperature of 22/17, 27/22 and 32/27°C). It was found that the photosynthetic rate was best expressed as leaf area basis. With this unit the photosynthetic rate of single leaves was closely related with chlorophyll content. There was linear relation between total N content (and protein) and chlorophyll content, indicating that photosynthesis per unit leaf area was closely correlated with nitrogen assimilation in leaves of the rice plants. Temperature and N level both affected photosynthesis of the rice leaves. In a tiller, the photosynthetic rate of leaves at different node positions was influenced by temperature and N level. At low temperature (22/17°C) there was no significant difference of photosynthesis among leaves at different node positions under different N treatments. However, when plants grew at higher temperatures, lower photosynthetic rate was found at lower-positioned leaves under low level of N, but not under higher levels of N. The results indicate that photosynthesis is under the control of N uptake and metabolism in rice plants.

Total soluble protein and amino-N increased with the increase in N concentration. There was higher N content (both soluble protein and total amino-N) in plants grown at lower temperature as compared with those of higher temperature growth plants, particularly with plants grown at higher levels of N, indicating the dilution effect of N by plant growth.

Ribulose biphosphate carboxylase (RuBPCase) and cytochrome C oxidase activities increased with the increase in the N level. However, these enzymic activities were low under all N treatments of plants grown at low temperature. Nitrate and nitrite reductase activities were enhanced by N concentration. No detectable

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activity of nitrate reductase was found in rice roots grown at N levels below 20 ppm N.

The increment of above-ground dry weight was closely correlated with N level and growth temperature. There was 2.7 fold increase in total dry weight in *japonica* rice and 3.3 fold in *indica* rice grown under high N level and at higher temperatures, as compared with those plants grown under low N and low temperature. However, leaf photosynthesis increased only 18% and 30% for *japonica* and *indica* rice, respectively. Growth analysis revealed that the effects of temperature and N treatments on rice growth were under the control of N metabolism and leaf growth. The results imply that N uptake is affected by temperature and N treatment. The growth of the rice plant was regulated by the uptake of N and subsequently the expansion of leaf growth which was governed by temperature under high N level. Thus, this study conformed our previous conclusion that the growth of the rice plant was regulated by the rate of nitrogen metabolism and subsequently by leaf growth.

**Key words:** Rice (*Oryza sativa*); nitrogen metabolism; growth temperature; ribulose biphosphate carboxylase; nitrate reductase; nitrite reductase; cytochrome C oxidase; photosynthesis.

### Introduction

Nitrogen is an important variable in manipulating cereal crops for high yields, and needs to be used efficiently. The dry matter production of rice plants is well known to be promoted by nitrogen fertilizer supply (Ishizuka, 1976; Murata, 1969; Yoshira, 1972). The effects of nitrogen on photosynthesis (Ishihara *et al.*, 1979; Oritani *et al.*, 1979; Makino *et al.*, 1983; Uchida *et al.*, 1982), ribulose biphosphate carboxylase (Makino *et al.*, 1983, 1984, 1985; Kabaki *et al.*, 1979) and nitrogen metabolism (Oji, 1974; Mae, 1986; Yoneyama, 1986) have been intensively studied in the rice plant. However, the effect of temperature on the relation between nitrogen utilization and growth is not very clear. Carbon and nitrogen acquisition and distribution are closely integrated in the growing plant (Steer, 1979; Hansen, 1980). Previously, we have reported the effect of nitrogen nutrition on photosynthesis and nitrogen distribution in the rice plant (Shieh and Liao, 1985). The effect of low temperature on the dry matter production and nitrogen distribution has been reported in pearl millet (Theodorides and Pearson, 1986) and white clover (Wolege and Suarez, 1983). In this present work the growth and photosynthesis of two rice cultivars were studied in the controlled environments to understand the interaction of temperature and nitrogen nutrition on plant growth in the rice plant.

### Materials and Methods

#### *Plant Culture and Growth Conditions*

Two rice (*Oryza sativa* L.) varieties, Tainung 62 (TN 62) (*japonica*) and Taichung Sen 3 (TCS 3) (*indica*) were used for experiments. The seeds were

sown on a stainless-steel screen which was floated on distilled water and germinated in darkness. After germination for three days in the dark, the plants were raised in water culture in an environment-controlled growth chamber under 16 h light period at day/night temperature of 27/22°C and relative humidity of 75±5%. Light was provided by a combination of fluorescent tubes and Metalarc (Sylvania metal-halide) lamps, giving a photosynthetic photon flux density of 300  $\mu\text{E s}^{-1}\text{m}^{-2}$  at plant height. The plants were given nutrient solution containing 20 ppm nitrogen (N). The nutrient solution was changed twice a week. Six rice seedlings at four-leaf stage were transplanted to an 1/5000 plastic pot and the treatments were commenced.

#### *Temperature and Nitrogen Treatments*

At the time of treatment, the pots were divided into three groups and transferred to controlled growth chambers at 22/17, 27/22 or 32/27°C. At each temperature regime, the plants were treated with five levels of N (5, 10, 20, 40, and 80 ppm N) of ammonium nitrate. The nutrient solutions were changed twice a week throughout the experimental period. Two sets of plants for each cultivar rice were prepared. One set was used for photosynthesis and enzyme assay. The other set was used for growth analysis and chemical analysis.

At the beginning and the end of N treatments, the plants were sampled. After measurements of plant height, number of leaves, leaf area and leaf fresh weight, the plants were separated into leaf blade, culm (stem+leaf sheath) and root portions and dried at 80°C in a draft oven, and then weighed. From the data of leaf area and dry weight of organs, growth analysis was carried out using the methods of Shieh and Liao (1985).

#### *Measurements of Photosynthetic Rate*

The upper three leaf blades of rice plants were used for measuring the photosynthetic rate and enzyme activity. Photosynthetic rate measurements were made using a Hansatech leaf disc oxygen electrode unit to trace the  $\text{O}_2$  exchange of leaves as described by Delieu and Walker (1981). Two leaf segments were cut from a leaf at the middle position by a leaf cutter (3.57 cm in diameter). The leaf segments were floated on 25 mM HEPES buffer (pH 7.0) containing 0.5 mM  $\text{CaCl}_2$ . Prior to the measurement of  $\text{O}_2$  exchange, the leaf segments were pre-illuminated with GE 300 PAR 56/2 MFL cool-beam incandescent lamps at 78.7  $\mu\text{E s}^{-1}\text{cm}^{-2}$  PAR for at least 30 min.  $\text{O}_2$  exchange was determined for the two leaf segments at 28°C. The oxygen concentration of 21% (v/v) of the atmosphere air was taken for calibration. The leaf segment was illuminated through the roof of the leaf disc chamber by means of a 250W-M5 quartz halogen lamp (EYE, Japan) with JAN-O housing, the light beam passing through a spherical flask containing

1%  $\text{CuSO}_4$  solution. The light intensity at the leaf surface was measured using a LI-190S Quantum sensor and LI-188B Quantummeter. The photosynthetic photon flux density was  $135 \mu\text{E s}^{-1} \text{cm}^{-2}$  PAR (400-700 nm). After  $\text{O}_2$  exchange measurements, one leaf segment was used to assay for chlorophyll content, and the other for total reduced nitrogen content. At least three tillers were made to measure  $\text{O}_2$  exchange rates.

#### *Enzyme Assays*

Assays of the enzyme activities were made on the leaves of different batches of tillers. Leaves of the three upper-most node positions were assayed. A sample of two leaves of the same node position was cut into segments and ground on ice with a motor and a pestle and small amount of sea sand in a buffer consisting of 25 mM HEPES (pH 7.5), 10 mM  $\text{MgCl}_2$ , 10 mM 2-mercaptoethanol, 1 mM  $\text{Na}_2\text{-EDTA}$  and 2% (w/v) PVP. The extract was filtered through  $80 \mu$  nylon net and centrifuged at 10000 xg for 10 min, and the supernatants were used directly to assay for enzymes activity. For analysis of nitrate and nitrite reductase activities, the leaf tissue was homogenized in 25 mM K-phosphate buffer (pH 8.8) containing 1 mM EDTA and 10 mM cysteine.

Ribulose-1, 5-bisphosphate carboxylase (RuBPCase) activity was determined by following  $^{14}\text{CO}_2$  incorporation into acid-stable products as described previously (Shieh and Liao, 1985). The reaction mixture consisted of 50 mM tricin buffer (pH 7.8), 20 mM  $\text{MgCl}_2$ , 5 mM DTT, 20 mM  $\text{NaH}^{14}\text{CO}_3$ , 1 mM RuBP and the enzyme extract in a total volume of 200  $\mu\text{l}$  at 30°C. The same extracts were also used to determine for chlorophyll, total free amino-nitrogen, and buffer-soluble protein. Two leaf samples were assayed for each node-positioned leaf. The leaf *in vitro* nitrate reductase assay was the procedure described by Neyra and Hageman (1975) and nitrite reductase was determined after Losada and Paneque (1971). Cytochrome C oxidase activity was assayed after Simon (1958). The crude enzyme extract used for RuBPCase assay was mixed with equal volume of Tween-100 and the enzyme activity was measured by following the decrease of optical density at 550 nm using an Uvikon 810 spectrophotometer connected to an Uvikon recorder 21.

#### *Nitrogen, Chlorophyll, Protein and Amino-nitrogen Analysis*

Total reduced nitrogen of the plant parts was determined in powdered samples as described (Shieh, 1978). Buffer-soluble protein was determined by Coomassie Brilliant Blue G-250 dye-binding method of Spector (1978) with bovine serum albumin as standard. Free amino-nitrogen was determined by the method of Yemm and Cocking (1955) using glycine as standard. Chlorophyll was determined after Wintermans and De Mots (1965).

**Results and Discussion**

*Dry Matter Production and Nitrogen Uptake*

The seedlings of rice varieties TN 62 and TCS 3 were treated with five levels of nitrogen ranging from 5 to 80 ppm N grown at three temperature regimes for a period of 3 weeks. Figure 1 shows the increments of dry weights of the above-ground straw, roots, culms and leaves. The growth of two rice varieties was affected by both temperature and nitrogen concentrations of culture media, and these two factors interacted to affect the response of the rice plants to nitrogen. The increase of above-ground dry weight showed saturation-type kinetics with nitrogen (Fig. 1a, b). However, the sharp of nitrogen response was temperature dependent, being that nitrogen demand increased with the increase in temperature and shifted the nitrogen saturation to higher nitrogen levels when rice plants were grown at higher temperatures. The nitrogen saturation in TN 62 was 20 ppm at 27/22°C, while TCS 3 was above 40 ppm at 32/27°C. The results indicated that the *indica* rice TCS 3 was more favour to grow under higher temperature condition and the aquisition of nitrogen was higher. On the other hand, the growth of above-ground (shoot) dry weigh in both rice varieties was reduced under all levels

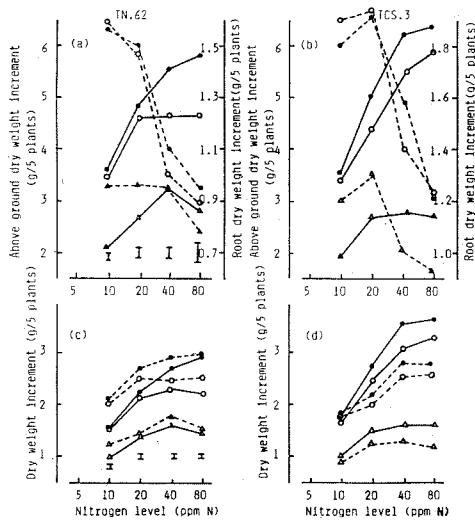


Fig. 1. Effect of nitrogen levels and growth temperature on the above-ground dry weight (solid lines) and root dry weight (dashed lines) (a, b), and leaf dry weight (solid lines) and culm dry weight (dashed lines) (c, d).  
 ●, 32/27°C; ○, 27/22°C; △, 22/17°C.  
 The vertical bars indicate the standard error of mean at each N level.

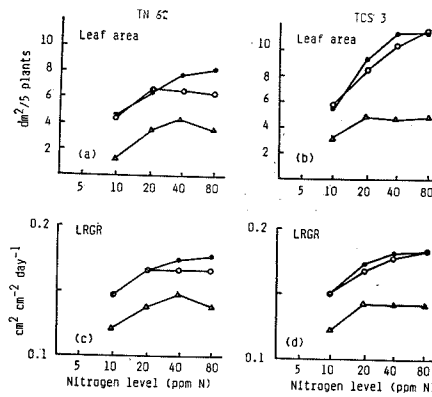


Fig. 2. Leaf area (dm<sup>2</sup>/5 plants) (a, b) and relative leaf area growth rate (cm<sup>2</sup> cm<sup>-2</sup> d<sup>-1</sup>) (c, d) of the two rice varieties under various growth conditions. The symbols are the same as in Fig. 1.

**Table 1.** Plant height, root length and root/shoot dry weight ratio at various nitrogen levels grown at different temperatures

Variety	N level (ppm)	Plant height (cm)			Root length (cm)			Root/shoot ratio (%)		
		22/17	27/22	32/27	22/17	27/22	32/27	22/17	27/22	32/27
TN 62	10	39.7	44.8	44.3	21.4	21.8	23.9	43	44	42
	20	40.5	52.1	52.0	23.1	22.8	23.4	35	31	30
	40	43.6	53.6	52.5	19.2	19.8	19.7	28	21	19
	80	42.6	52.8	53.5	21.2	21.3	21.1	27	19	16
TCS 3	10	30.5	38.0	38.0	24.7	24.3	24.0	60	59	49
	20	32.7	39.7	40.4	24.0	25.9	25.7	45	43	40
	40	32.2	42.1	44.1	22.0	20.0	21.6	35	26	25
	80	32.2	42.2	44.6	23.4	21.4	23.0	33	21	19

of nitrogen concentrations with plants grown at 22/17°C. Figure 1a and 1b also show that nitrogen utilization in TCS 3 at low temperature (22/17°C) was suppressed, as TCS 3 was saturated at 20 ppm N while the dry weight increments of TN 62 at the same temperature was saturated at 40 ppm N. The distribution of dry weight in shoots showed variety difference and temperature dependence (Fig. 1c, d). At low level of nitrogen, the distribution of dry weight between leaf and culm was approximately equal at all temperature treatments, while more dry weight was distributed to leaves at higher levels of nitrogen at higher temperature.

On the other hand, the plants grown at low nitrogen levels and low temperature tended to produce less dry weight than those supplied with higher levels of nitrogen and distributed a greater proportion of dry weight to their roots (Fig. 1a, b; Table 1). The effect of nitrogen nutrition on the distribution of dry weight between shoots and roots was similar to the previous results (Shieh and Liao, 1985). As shown in Fig. 1a and 1b, the root dry weight declined sharply with the increase in nitrogen concentrations of the culture media at higher temperatures in the two rice varieties. With limited nitrogen the plants formed few leaves and more roots. This phenomenon was further enhanced by low temperature (Woledge and Suzrez, 1983). Table 1 shows the effects of growth temperatures and nutrient nitrogen concentrations on plant height, root length, and the dry weight ratio of root/shoot. At low temperature, the growth of plant height decreased, while the root length was similar in all nitrogen treatments. The root/shoot ratios decreased from 43% at 10 ppm N and 22/17°C to 16% at 80 ppm N and 32/27°C for TN 62; from 60% to 19% for TCS 3. It clearly showed that the *indica* cv. TCS 3 distributed more dry weight to roots at low temperature and low nitrogen level than *japonica* cv. TN 62 did. The results indicated that the nitrogen uptake was probably limited by low temperature and the plants invested more dry

weight to the roots for growth. As the growth temperature increased, the uptake of nitrogen was not rate limiting and the nutrient nitrogen concentration became rate limiting to nitrogen uptake and utilization for plant growth.

Figure 2 shows the leaf growth as affected by the growth conditions. The growth of leaf area was affected by the interaction of temperature and nitrogen nutrition. Low temperature decreased the leaf expansion and TCS 3 could not efficiently use of nitrogen at low temperature as TN 62 did. No more response of leaf expansion in TCS 3 to nitrogen when nitrogen level was above 20 ppm N, while leaf expansion in TN 62 increased up to 40 ppm N at low temperature. On the other hand, the response of leaf expansion in TCS 3 at higher temperature was greater than that of TN 62. The relative leaf growth rate (RLGR) showed near nitrogen saturation, but it was higher at 32/27 or 27/22°C than at 22/17°C (Fig. 2a, b).

The total amount of nitrogen accumulation in shoots increased with the increase in external nitrogen concentrations (Fig. 3a, b), but it was higher at 32/27 or 27/22 than at 22/17°C. The effects of various treatments on the total amount of nitrogen in shoots were similar to those of shoot dry weight. The response curves to temperature indicated increased dependent on nitrogen up to

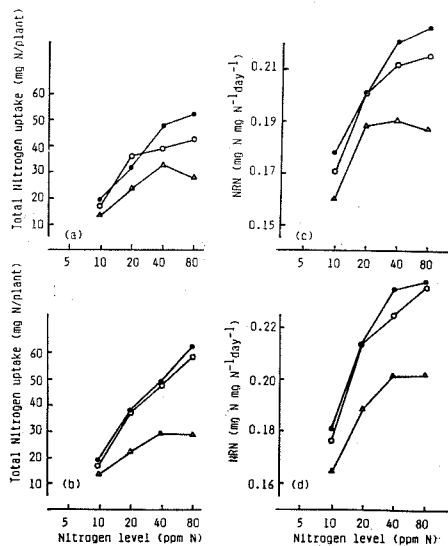


Fig. 3. Effects of nitrogen levels and growth temperature on the total amount of N uptake (a, b) and relative nitrogen accumulation rate (c, d) in above-ground parts of rice plants var. TN 62 (a, c) and TCS 3 (b, d). The symbols are the same as in Fig. 1.

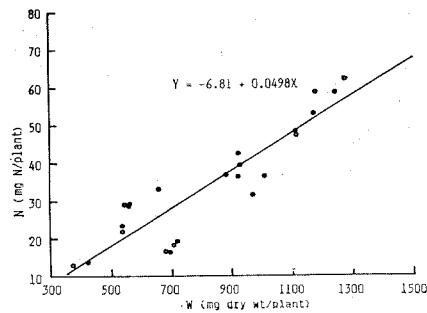


Fig. 4. Correlation between dry weight increment (W) and nitrogen accumulation (N) in TN 62 (closed symbols) and TCS 3 (open symbols).

**Table 2.** Nitrogen contents (%N) and amount of total nitrogen uptake (mg N/plant) of plant parts in various treatments

Var.	N level (ppm)	Leaf			Stem+leaf sheath			Root			
		22/17	27/22	32/27	22/17	27/22	32/27	22/17	27/22	32/27	
TN 62	% N	10	4.24	3.46	4.17	2.28	1.60	1.68	2.31	1.45	1.44
		20	5.44	4.92	4.41	3.33	3.16	2.32	2.94	2.85	1.97
		40	6.04	5.08	5.35	3.96	3.43	3.43	3.52	3.22	3.03
		80	6.20	5.71	5.43	4.10	3.61	3.63	3.59	3.72	3.37
	mg N per plant	10	8.5	10.4	12.9	5.6	6.5	7.0	4.48	4.62	4.51
		20	15.0	21.3	19.8	9.4	15.7	12.4	5.76	8.33	5.92
		40	19.9	23.1	28.9	13.9	16.8	19.9	6.64	6.40	6.70
		80	17.4	25.3	31.7	12.3	17.9	21.8	5.58	6.69	6.41
TCS 3	% N	10	4.19	3.54	3.65	2.45	1.55	1.71	2.25	1.37	1.49
		20	4.47	4.97	4.49	3.46	3.15	2.48	2.82	2.67	1.63
		40	5.69	5.33	5.62	4.24	3.19	3.54	3.72	3.22	3.25
		80	5.90	6.01	5.71	4.52	3.63	3.70	4.01	3.77	3.76
	mg N per plant	10	8.9	11.7	12.6	4.5	5.5	6.2	5.44	5.26	5.36
		20	13.6	24.6	24.9	8.8	12.8	12.2	7.50	10.12	6.79
		40	18.2	33.0	39.3	11.0	16.3	20.0	7.55	9.19	10.36
		80	18.3	39.9	41.8	11.3	19.4	20.9	7.47	9.32	9.21

40 ppm N in TN 62 and 80 ppm N in TCS at higher temperatures. There was a close relation between dry weight increments and amounts of nitrogen accumulation. As shown in Fig. 4, the relation between dry weight increment (W, mg/plant) and nitrogen accumulation (N, mg N/plant) showed significant linear correlation. The regressions for the two rice varieties and all data pooled were given by:

$$\text{TN 62: } N = -3.9682 + 0.0452W \quad (r = 0.858, n = 12)$$

$$\text{TCS 3: } N = -7.7701 + 0.0516W \quad (r = 0.927, n = 12)$$

$$\text{Pooled: } N = -6.8091 + 0.0498W \quad (r = 0.896, n = 24)$$

The results demonstrated that the growth of rice plants was well coupled with the ability of plants to take up and utilize nitrogen.

Table 2 shows the nitrogen contents (N%) and amounts of nitrogen accumulation (mg N/plant) in different treatments of nitrogen and temperatures. The magnitude of nitrogen content in the plant parts was in the order of leaves > culms > roots. Nitrogen content of leaves, culms and roots increased with the increase in N concentrations. Leaf nitrogen contents were not different in plants treated with nitrogen above 20 ppm at higher temperatures, while the accumulation of nitrogen in leaves increased with the increase in temperature and N levels.



At low temperature, there were higher nitrogen contents in leaves and culms as compared with those at higher temperatures. The nitrogen contents in roots were low at low nitrogen levels (10, 20 ppm N), particularly when plants were grown at higher temperatures. It indicated that the translocation of nitrogen from root to shoot was inhibited by low temperature. The results indicated the response of plant to nitrogen was temperature dependent and the temperature-dependent nitrogen response might be different between the plants growing at low and higher temperatures. Both uptake and utilization of nitrogen may limit plant growth in a way or another dependent upon the growth environments. There was a dilution effect of growth on nitrogen contents when plants were growing at higher temperatures. This can be seen from the accumulation of nitrogen in plants. At low level of N, the amount of nitrogen in shoots at higher temperature was 40 to 50% higher than that at low temperature, while it increased 1.5 to 2.0 folds in TN 62 and 2 to 2.3 folds in TCS 3 when treated with high levels of nitrogen, but the amount of nitrogen in roots showed little different in all treatments. Most of nitrogen taken up by roots was translocated to the top, particularly in support of leaf growth. Table 3 shows the variance analysis of nitrogen accumulation in leaves of the two rice varieties. There clearly showed interaction of nitrogen and temperature on nitrogen utilization in leaves and the difference in variety response.

*Relative Growth Rate and Relative Nitrogen Accumulation Rate*

The relative growth rate (RGR) of the two rice varieties showed a saturation-

**Table 3.** Analysis of variance for amount of nitrogen accumulation (mg N/plant) of rice leaves

	df	MS	F
Block (B)	1	0.3333	0.128
Variety (V)	1	231.44	88.56**
Ea	1	2.61	
Temperature (T)	2	577.32	159.78**
V × T	2	71.18	19.70**
Eb	4	3.61	
Nitrogen level (N)	3	815.25	544.38**
V × N	3	39.25	26.94**
T × N	6	53.13	36.12**
V × T × N	6	11.96	8.13*
Ec	18	1.471	
Total	47		

\*,\*\*: Significant at 5% and 1% level, respectively.

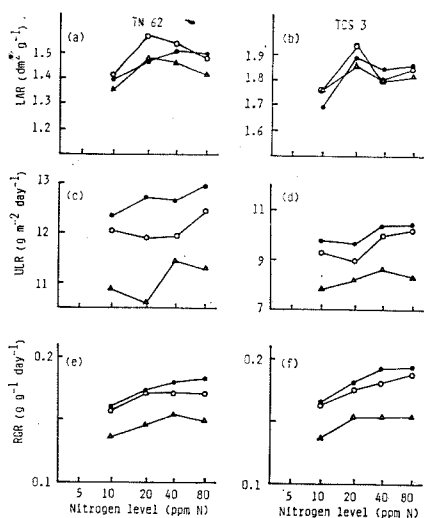


Fig. 5. Leaf area ratio (LAR), unit leaf rate (ULR) and relative growth rate (RGR) under various growth conditions. The symbols are the same as in Fig. 1.

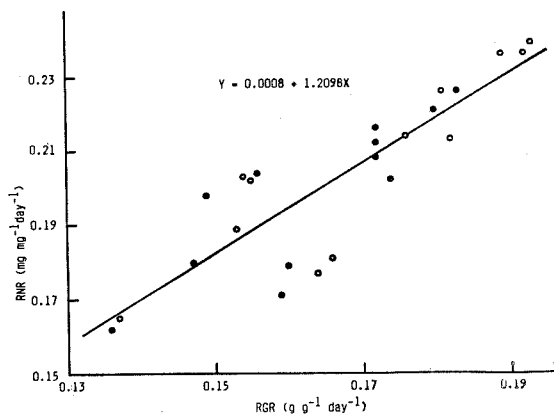


Fig. 6. Relationship between relative nitrogen accumulation rate (RNR) and relative growth rate (RGR) in TN 62 (closed symbols) and TCS 3 (open symbols).

type growth kinetics as response to external nitrogen concentrations at all temperatures tested (Fig. 5e, f). RGR were similar at 27/22°C and 32/27°C, but decreased at 22/17°C. Nitrogen concentrations had little effect on the unit leaf rate (ULR), while ULR was significantly affected by growth temperature (Fig. 5c, d). In a whole, ULR at high N level and higher temperature was 18% higher in TN 62 and 30% in TCS 3 than it at low temperature and low nitrogen levels, when dry weight increased 2.7 fold in TN 62 and 3.3 fold in TCS 3. The contribution to the shoot dry weight was mainly due to the difference in leaf area growth, being 3.2 and 3.8 folds increase in leaf area in TN 62 and TCS 3, respectively (Fig. 2a, b). Therefore, the growth of rice plants was regulated via the ability of nitrogen utilization and its effect on leaf growth. The results further extended the conclusion suggested in the previous finding (Shieh and Liao, 1985) that the growth of the rice plant is controlled by the rate nitrogen uptake and subsequently by leaf area growth. The leaf area ratios (LAR) was smaller at low N level and low temperature. Similar response of LAR to nitrogen concentration was shown in all temperature treatments (Fig. 5a, b).

The relative nitrogen accumulation rates (RNR) calculated from  $RNR = \ln(N_2 - N_1) / (t_2 - t_1)$ , where N is amount of nitrogen accumulation at time  $t$ , was near nitrogen saturation at all temperature tested (Fig. 3c, d). RNR was higher at 27/22 and 32/22 than at 22/17°C. Correlations between RNR and RGR were highly

significant. The regressions for the two rice varieties and all data (Fig. 6) were given by:

$$\text{TN 62: RNR} = 0.0032 + 1.1944 \text{ RGR} \quad (r=0.837, n=12)$$

$$\text{TCS 3: RNR} = -0.0003 + 1.2170 \text{ RGR} \quad (r=0.880, n=12)$$

$$\text{Pooled: RNR} = 0.0008 + 1.2098 \text{ RGR} \quad (r=0.896, n=12)$$

However, there was deviation of plant response to nitrogen at different temperatures in the two rice varieties. The curve of RNR against RGR in TN 62 at low temperature was concave, while it was convex in TCS 3 and linear at higher temperatures (Fig. 7). The relation between RNR and RGR might indicate that at the whole-plant level, a coupling between whole-plant growth and nitrogen uptake when nitrogen supply is not limiting. Such a coupling, however superficial, should be useful when mathematically modelling whole-plant carbon and N acquisition (Theodorides and Pearson, 1981).

*Chlorophyll Content and Photosynthesis*

Temperature had little effect on chlorophyll contents of leaves, while nitrogen concentrations below 20 ppm N caused a decrease in chlorophyll (Fig. 8a, b). Leaf chlorophyll contents were closely correlated with leaf nitrogen and protein contents (data not shown). Figure 9 shows a typical measurement of photosynthetic oxygen

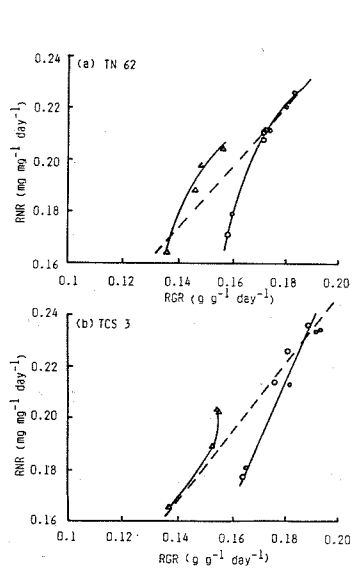


Fig. 7. Relationship between relative nitrogen accumulation rate (RNR) and relative growth rate in TN 62 (a) and TCS 3 (b). The symbols are the same as in Fig. 1.

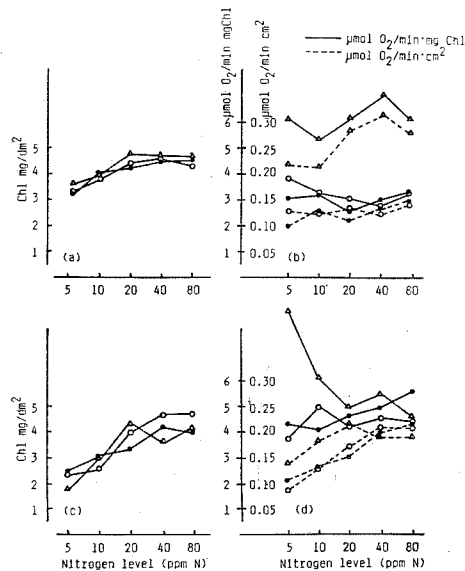


Fig. 8. Chlorophyll content (a, c) and photosynthetic O<sub>2</sub> evolution (b, d) in TN 62 (a, b) and TCS 3 (c, d) under various growth conditions. The symbols are the same as in Fig. 1.

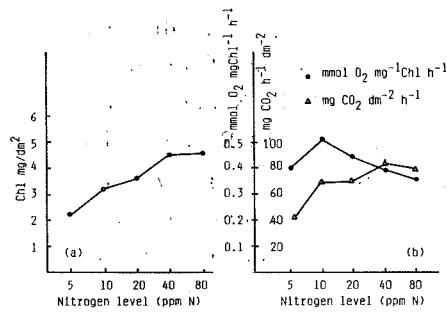


Fig. 9. Chlorophyll content (a) and photosynthesis (b) of TCS 3 under various N treatments at 27/22°C.

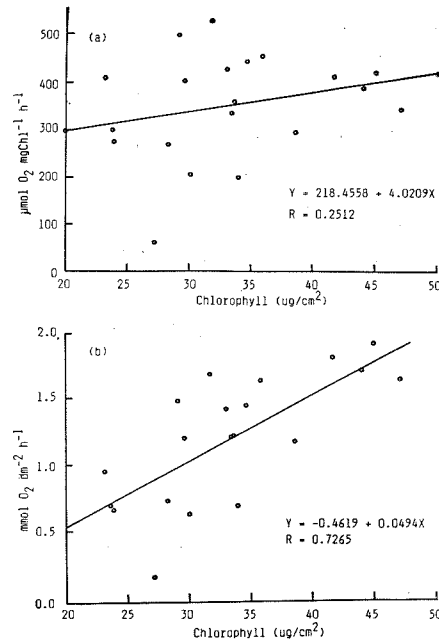


Fig. 10. Relationship between photosynthetic  $O_2$  evolution on chlorophyll (a) and leaf area (b) basis and leaf chlorophyll content.

evolution at the youngest fully-expanded leaves in TCS 3 grown at 27/22°C. Chlorophyll contents on basis of leaf area increased with the increase in nitrogen concentration up to 40 ppm N (Fig. 9a). Figure 9b shows that photosynthetic rates based on chlorophyll content were not well related with nitrogen treatments, or higher rates were found with those leaves under low N levels. When based on unit of leaf area photosynthetic rates saturated at N supply up to 10 ppm N. These findings were consisted with the previous results (Shieh and Liao, 1985) that photosynthetic rate was closely related with leaf nitrogen content (Table 2) irrespective of nutrient treatment, environments and leaf age (Evans, 1983). Figure 10 shows the relation of photosynthetic rate in chlorophyll unit (Fig. 10a) and in leaf area unit (Fig. 10b) with chlorophyll content. It can be seen clearly that the photosynthetic parameters were best expressed on leaf area basis to show meaningful comparison between treatments. Figures 8c and 8d show the photosynthetic rates under various treatments of nitrogen and temperature. Higher photosynthetic rates were found with plants grown at low temperature, while dry weight increments were greater with plants grown at higher nitrogen levels and higher temperatures (Fig. 3a, b). The reason is the effect of temperature on leaf area expansion (Fig. 2a, b). Growth temperature and N supply may interact

to regulate the rate of photosynthesis in individual leaves. Figure 11 clearly demonstrates this implication. As shown in the figure, photosynthetic rates of the upper three leaves of a tiller were measured. Decrease of external nitrogen concentration accelerated the senescence of old leaves at higher temperatures, but not at low temperature growth plants. However, when the plants were supplied with sufficient nitrogen, the photosynthetic rates of the leaves were not different at all temperatures tested. A depletion of N supply may have triggered the mobilization of N from the older leaves for the growth of young tissues (Blacklow and Incoll, 1981; Mae *et al.*, 1983; Shieh and Liao, 1985). It was more pronounced when plants growth was rapid at higher temperature or nitrogen supply was rate limiting.

*Soluble Protein, Amino-N, Nitrate and Nitrite Reductase*

Figure 12 shows the effects of various treatments on buffer-soluble protein and amino acid-N contents and nitrogen reduction enzymes, nitrite reductase (NRA) and nitrite reductase (NiRA) activities. Amino-N and soluble protein increased with the increase in nitrogen concentration. Protein and amino-N contents were higher at low temperature in both rice varieties. It could be either low temperature inhibited nitrogen uptake and translocation or plant growth. At higher temperature the dilution effect of growth might decreased the contents of nitrogen. NRA and NiRA decreased at N levels below 20 ppm. NRA in TCS 3 showed higher activities at all levels of N and NiRA above 40 ppm N, indicating that nitrogen metabolism in the *indica* rice more adapted to higher temperature. The activities

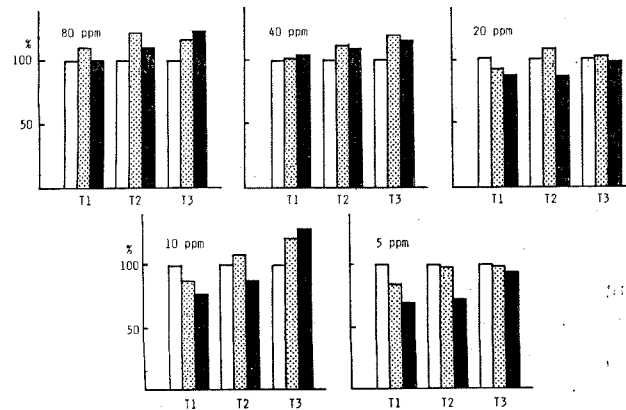


Fig. 11. Effect of nitrogen concentrations and temperature on the photosynthetic oxygen evolution in upper three leaves of TN 62. T1, 32/27; T2, 27/22; T3, 22/17°C. □, upper-most leaf; ▨, second leaf; ■, third leaf, counted down from the top.

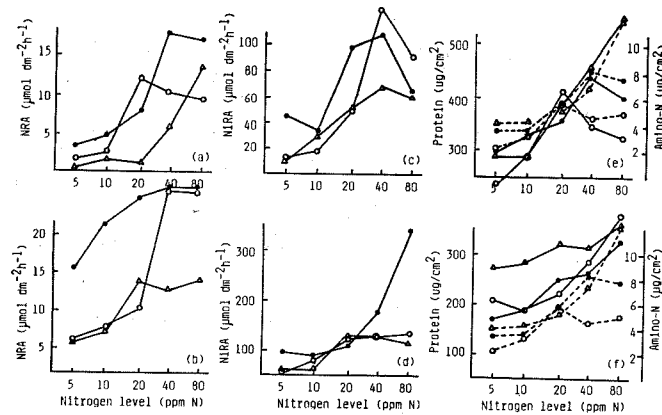


Fig. 12. Nitrate reductase (a, b), nitrite reductase (c, d), soluble protein (solid lines) and amino-N (dashed lines) (e, f) of leaves in rice var. TN 62 (a, c, e) and TCS 3 (b, d, f) under various growth conditions. The Symbols are the same Fig. 1.

of NRA and NiRA were closely related with protein contents in leaves.

The protein content, amino-N content, and NRA and NiRA activities were also measured in various parts of plants (data not shown). The amino-N contents were in the order of leaves > culms > roots. Protein contents in leaves was 2 to 3 folds and 6 to 8 folds higher than those of culms and roots, respectively. NRA and NiRA were the highest in leaves. No detectable NRA in roots were found when plants were growing at N levels below 20 ppm N.

#### *Ribulose Bisphosphate Carboxylase and Cytochrome C Oxidase*

Figure 13 shows the ribulose bisphosphate carboxylase (RuBPCase) and cytochrome C oxidase activities. RuBPCase activity increased with increase in nitrogen concentrations (Shieh and Liao, 1985). Kabaki *et al.* (1979) also showed the decrease of RuBPCase activity when N was depleted from the culture solution. However, RuBPCase activities showed variety different in response to temperature (Fig. 13a, b). At low temperature RuBPCase activities did not response to the increase in nitrogen concentration of culture solutions. On the other hand, RuBPCase activities were higher at lower temperatures at all N levels. Nevertheless, the response of RuBPCase to nitrogen and temperature treatments was in agree with the changes of photosynthetic rates in the rice leaves (Fig. 8). Similar results were also reported (Shieh and Liao, 1985; Makino *et al.*, 1983).

The mitochondria enzyme cytochrome C oxidase activities were increased with the increase in nitrogen levels as in RuBPCase activity. Similar to RuBPCase, cytochrome C oxidase activity in TN 62 was higher at low temperature and was not different in all N levels at 32/27°C, while cytochrome C oxidase activities in

TCS 3 were higher at 27/22 or 32/22 than at 22/17°C. Since photorespiration is closely related with mitochondria metabolism in the light. Nitrogen treatments may affect photorespiration. In this context, increase of nitrogen in leaves may stimulate photorespiration. Kabaki *et al.* (1979) reported N depletion lowered RuBPCase as well as RuBP-oxygenase, but RuBPO/C ratio increased. It was in contrast with our early finding (Shieh and Liao, 1985) and others (Fritsch and Jung, 1984; Hall and Keys, 1983; Peoples *et al.*, 1980). Whether the effect of temperature on cyochrome C oxidase is oxidative respiration or photorespiration needs further study.

### Conclusioning Remarks

The results in the present studies demonstrates the close relation between plant growth of rice plants and nitrogen acquisition. From the study of RNR and RGR it clearly shows that daily plant growth may be sufficiently supported by nitrogen accumulation if the nitrogen is not rate limiting. However, this modelling does not distinguish among nitrogen uptake, reduction and metabolism, as shown by the present studies that the effects of environmental factors may impose different stresses on the utilization of nitrogen by plants. There also has variety difference in response to nitrogen nutrition and temperature. The study also demonstrate that single leaf photosynthesis is not sufficiently enough to estimate the photosynthetic capacity at the whole-plant level.

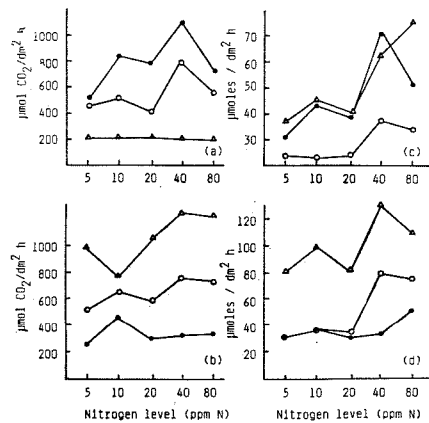


Fig. 13. RuBPCase activity (a, b) and cytochrome C oxidase activity (c, d) in TN 62 (a, c) and TCS 3 (b, d) under various growth conditions. The symbols are the same as in Fig. 1.

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## 生長溫度與氮素營養對水稻光合作用及 氮素代謝的影響

謝 昱 暉 廖 文 光

中央研究院植物研究所

兩個水稻品種臺農62號及臺中秈3號的秧苗在四葉齡時以 5~80 ppm N 等五種濃度的硝酸銨在日/夜溫 22/17、27/22、及 32/27°C 的溫度下處理三個星期，取樣調查植株的乾物重、葉面積、葉綠素含量、全氮量、可溶性蛋白質含量、光合作用速率、以及酵素活性，以期瞭解溫度與氮素的相互作用對水稻的生長與光合作用的影響。水稻的生長受到生長溫度與溶液氮素濃度的交感作用呈飽和型的動力生長曲線，溫度與氮素濃度分別影響水稻對氮素及生長溫度的反應，並且品種間的反應亦有差異。

水稻的根長及根乾重隨氮濃度增加而遞減，但地上部的乾重正相反，指示在缺氮下，乾物質的分配向根部的分配增加，以促進氮之吸收。在 20 ppm N 以下，根硝酸還原酵素活性已不能測到。

光合作用以葉面積為單位時隨氮的提高而增加，但品種間對溫度的反應有差別，顯示秈稈稻的光合作用，對溫度的適應性不相同，稈稻品種較耐低溫，而秈稻品種較耐高溫。光合作用速率與全氮量及葉綠素含量成正相關。光合作用與葉部的雙磷酸核酮糖羧化的關係密切，同受溫度與氮肥的影響，趨勢一致。

本試驗的結果提示水稻的生長在營養生長期受到氮吸收及其與溫度的交感控制葉面積的生長及光合作用，進而影響水稻乾物質的生產以及碳與氮的分佈。