

EFFECT OF NITROGEN ON GROWTH AND  
PHOTOSYNTHESIS OF RICE PLANTS  
(*ORYZA SATIVA* L.)<sup>1,2</sup>

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**Abstract**

The four-leaf-stage seedlings of four rice cultivars were grown with various concentrations of ammonium nitrate nitrogen ranging from 5 to 120 ppm N for three weeks. Plants showed saturation-type growth kinetics with nitrogen but the shape of the nitrogen response curve was dependent on rice cultivars. Nitrogen uptake was closely correlated with dry weight increment. Growth analysis revealed that low levels of nitrogen inhibited mainly leaf area growth, with net assimilation rate affected only at more severe nitrogen stress. Net photosynthetic carbon dioxide uptake was positively correlated with transpiration and specific leaf weight. Chlorophyll and soluble leaf protein contents were positively related with leaf nitrogen content and they were higher at elevated levels of nitrogen. However, net carbon dioxide exchange showed little difference among nitrogen treatments, except at more severe nitrogen stress. Activities of ribulose-1,5-bisphosphate carboxylase, phosphoenolpyruvate carboxylase, nitrate reductase and nitrite reductase in the leaf increased with increasing in external nitrogen concentration. However, the specific activity of RuBPCase and the ratio of RuBP oxygenase to RuBPCase remained fairly constant irrespective of nitrogen treatment. The results indicate that the growth of the rice plant is controlled by the rate of nitrogen uptake and subsequently by leaf area growth.

**Key words:** Rice (*Oryza sativa* L.); nitrogen stress; leaf area growth; net assimilation rate; ribulose bisphosphate carboxylase; RuBP oxygenase/RuBPCase ratio; phosphoenolpyruvate carboxylase; nitrate reductase; nitrite reductase.

**Introduction**

Light, temperature and mineral nutrients are the major environmental factors which determine growth and dry matter productivity of crop plants. In agricultural

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practice the factor mineral nutrition is routinely manipulated to increase yield and to control quality of crop. The significance of these nutrients on the yield of crops and the underlying physiological processes has been studied intensively (cf. Mengel and Kirkby, 1978; Moorby and Besford, 1983). Nitrogen fertilizer strongly affect plant growth and dry matter productivity. It has been found that photosynthesis is strongly influenced by the nutrient status, especially the nitrogen level, of cereal crops (Fritsch and Jung, 1984; Thomas *et al.*, 1978; Imai and Murata, 1978). Nitrogen can influence photosynthesis (hence growth) through affecting leaf area itself or through changes in rate per unit leaf (net assimilation rate, NAR). After many years of study, though, the relationship of these characters to each other and to productivity remains confusing and controversial. Some workers have suggested that growth limitation by low N results mainly from decreased photosynthesis rate, while other effects are secondary (Natr, 1975; Bolton and Brown, 1980). Watson (1952, 1963) concluded that changes in the nutrient supply affect the yield of plants by changing the size of the photosynthetic system, with little or no effect on its efficiency (NAR) (Bouma and Dowling, 1966; Halse *et al.*, 1969; Imai and Murata, 1978; Motta and Medina, 1978). Others have shown that growth responses to changes in N supply were reflected in effects on the net assimilation rate as well as on leaf growth (Bouma, 1970; Radin, 1983).

In the rice plant, the effects of N on leaf photosynthesis (Ishihara *et al.*, 1979; Makino *et al.*, 1983; Oritani *et al.*, 1979; Uchida *et al.*, 1980, 1982; Yoshida and Coronel, 1976) and dry matter production and yield (cf. Ishizuka, 1976; Murata, 1969; Yoshida, 1972; Shieh, 1978) have been studied intensively. In this paper, we report the effects of a series levels of N on growth dry weight accumulation, contents of total reduced N and soluble leaf protein, and enzymatic activities.

## Materials and Methods

### *Plant Material and Growth Conditions*

Four cultivars of rice (*Oryza sativa* L.) plants, Tai-Nung 62 (TN 62), and Todokiwase (TWS) (*japonica*), Chia-Nung Sen 11 (CNS 11) and Taichung Sen 3 (TCS 3) (*indica*), were used. The rice seeds were sown on a stainless-steel screen which was floated on distilled water and germinated in darkness. After germination, the rice plants were grown in water culture in a green-house. For those plants grown in winter, the light intensity was supplemental with 400-W mercury lamps (Sylvania H33G1. 400/DX). The rice seedlings at four-leaf stage were transplanted to 1/5,000 plastic pots containing vermiculite and nutrient solution with different nitrogen (N) concentrations.

### *Nitrogen Treatment Experiment*

The four-leaf stage rice seedlings were transplanted to give six plants a pot.

The plants were treated with six levels of N (5, 10, 20, 40, 80 and 120 ppm N) of ammonium nitrate. The nutrient solution was changed twice a week throughout the experimental period. Two sets of plants of each rice cultivar 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 harvested. After measurements of the plant height, the number of leaves, leaf area, leaf fresh weight and the tiller number, the plants were separated into leaf blade, culm (stem+leaf sheath) and root portions and dried at 80°C in a drift oven, and then weighed. From the data of leaf area and dry weight of organs, growth analysis was carried out using the method of Shieh (1977).

#### *Gas Exchange Measurements*

The youngest fully-expanded leaves were used to measure gas exchanges. Rates of CO<sub>2</sub> exchange and water vapor exchange were determined using an open-circuit system in a water-jacketed leaf chamber at 30°C. Rates of CO<sub>2</sub> exchange were determined using an infrared CO<sub>2</sub> analyser (IRGA, Beckman model 852) and water vapor was determined using a dew-point hygrometer (General Eastern system 1100 DP, USA). The IRGA was calibrated with standard CO<sub>2</sub> of known concentration obtained from Matheson, USA.

#### *Illumination*

The leaf chamber was illuminated by means of two 400-W Sylvania Metalarc Lamps, the light beam passing through a Plexiglas tank of water of path-length 10 cm. The light intensity was measured using a LI 190S Quantum Sensor and LI-185A Quantum meter. The photosynthetic photon flux density was 570  $\mu\text{E}/\text{m}^2 \cdot \text{s}$ .

#### *Enzyme Assays*

Assays of the enzymes activity were made on the leaves used in the gas exchange experiments, or of similar age and position on the rice plant to those used in the gas exchange experiments. After the measurements of leaf fresh weight and leaf area, the leaf blade was cut into segments and ground on ice with a motor and a pestle and a small amount of sea sand in a buffer containing 50 mM HEPES (pH 7.5), 5 mM MgCl<sub>2</sub>, 5 mM 2-mercaptoethanol (2-ME), 1 mM Na<sub>2</sub>-EDTA and 2% PVP-40. Homogenates were centrifuged at 10,000 xg for 10 min and the supernatants were used directly for enzyme assays. For analysis of nitrate and nitrite reductase activities, the leaf tissue was homogenized in 25 mM K-phosphate buffer (pH 8.8), 1 mM Na<sub>2</sub>-EDTA and 10 mM cysteine.

The following enzymes were assayed as previously described: ribulose bisphosphate (RuBP) carboxylase (Lorimer *et al.*, 1977), RuBP oxygenase (Kabaki *et al.*,

1979), PEP carboxylase (Holaday *et al.*, 1977), RuBP carboxylase was assayed using  $H^{14}CO_3$ -based assay. The spectrophotometric assay was made for phosphoenolpyruvate (PEP) carboxylase. 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).

#### *N, P, K, Protein, and Chlorophyll Contents*

Total reduced N of the plant parts was determined in the powdered samples as described (Shieh, 1978). Buffer soluble protein in the extracts was determined by Coomassie blue G-250 dye-binding method of Spector (1978) with bovine serum albumin as a standard. Phosphorus was determined by the method of Lazarus and Chou (1972). Potassium was determined as described (Shieh, 1976). Chlorophyll was determined after Winterman and De Mots (1965).

### Results and Discussion

#### *Whole Plant N Content and Dry Weight*

The seedlings of the four rice cultivars were treated with six levels of nitrogen (N) ranging from 5 to 120 ppm N for a period of 3 weeks grown in a green house. Table 1 shows the influences of N levels on the plant height, tiller and leaf numbers, leaf area and the above-ground shoot dry weight. The plant height was shorter when grown at N level below 20 ppm N. No difference in plant height was found between 20 to 120 ppm N. Leaf area (Table 1) and shoot dry weight (Table 1 and Fig. 1a) increased with increase of N concentration up to 40 ppm N, while

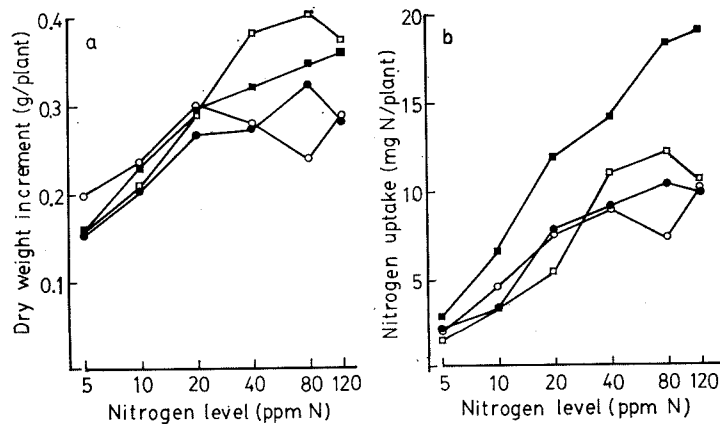


Fig. 1. Dry weight accumulation(a) and total nitrogen uptake(b) of the four rice cultivars grown at six levels of N nutrition. In this and subsequent figures, ○—○: TN 62; ●—●: TWS; □—□: TCS 3; and ■—■: CNS 11.

**Table 1.** *Plant height, number of tillers and leaves, leaf area, and total dry weight, at final harvest for exponentially growing rice plants grown at six levels of nitrogen concentrations*

Two replicates with eight plants each were used for each treatment. A 'time zero' harvest took place at the time the plants were transferred to the nitrogen treatments; These were the seedlings at the four-leaf stage grown at 40 ppm N.

Cultivar	Nitrogen levels (ppm N)	Plant height (cm)	Number of		Leaf area (cm <sup>2</sup> )	Total dry weight (mg/plant)
			tillers	leaves		
Tainung 62 (TN 62)	Start	30.5	0	3.6	9.4	34
	5	48.2	0	4.9	32.3	188
	10	49.0	0	5.3	42.3	230
	20	59.2	0.14	6.0	55.3	301
	40	60.2	0.5	6.6	62.7	330
	80	60.1	1.1	7.7	69.0	358
	120	59.2	0.56	6.9	65.5	316
Todokiwase (TWS)	Start	29.3	0	3.2	9.7	32
	5	48.5	0	4.8	38.6	232
	10	50.1	0	5.3	51.0	269
	20	57.7	0	5.9	64.9	333
	40	55.4	0.37	6.2	62.2	311
	80	54.5	0.22	5.6	59.1	271
	120	51.8	0.94	7.3	67.9	322
Chianung Sen 11 (CNS 11)	Start	28.2	0	3.2	13.6	46
	5	30.4	0	4.2	33.5	205
	10	43.5	0.69	6.2	55.9	272
	20	43.5	0.44	6.9	64.3	340
	40	42.4	2.0	10.6	101.8	339
	80	43.0	2.0	11.0	111.1	390
	120	45.8	2.0	10.8	110.0	406
Taichung Sen 3 (TCS 3)	Start	29.8	0	3.3	14.6	48
	5	42.5	0	4.8	36.0	204
	10	42.7	0	5.6	48.9	257
	20	49.9	0.06	5.8	66.5	335
	40	56.9	0.38	7.4	97.6	429
	80	54.1	1.0	8.6	103.9	449
	120	54.8	0.6	8.0	94.8	421

the early-mature variety Todokiwase (TWS) was saturated at 20 ppm N. Lower the N concentration of the culture media caused the senescence of lower-positioned leaves of a plant in all four rice varieties.

Nitrogen uptake by rice plants was sigmoidus as in dry weight. No further uptake of N was found when N level in the culture solution was above 40 ppm N, except 80 ppm N for Chia-Nung Sen 11 (CNS 11), an *indica* rice (Fig. 1b). The dry weight increments of the rice plants were closely related with the total amount of N uptake. As shown in Fig. 2, the relationship between dry weight increment (W) and N uptake (N) showed significantly linear correlation. The regression for all data pooled and four rice cultivars were given by:

$$\text{Pooled: } W = 0.1771 + 0.0118 N \quad (r = 0.8353, n = 24)$$

$$\text{TN 62: } W = 0.1304 + 0.01667 N \quad (r = 0.9799, n = 6)$$

$$\text{TWS: } W = 0.1805 + 0.0124 N \quad (r = 0.8599, n = 6)$$

$$\text{CNS 11: } W = 0.1408 + 0.01182 N \quad (r = 0.9862, n = 6)$$

$$\text{TCS 3: } W = 0.138 + 0.0221 N \quad (r = 0.9911, n = 6)$$

The results showed that the growth of the rice plants was well coupled with the ability of plants to take up nitrogen as shown in pooled regression line and it was influenced by the application of external N. However, Fig. 2 also shows variety difference in response to the external N concentration, being that cultivar Taichung Sen 3 (TCS 3) was most sensitive to external N, and CNS 11 was more adaptive to heavy N application under these green-house growth conditions.

#### *Effect of N Treatment on Dry Weight Partitioning and N, P, K Contents*

Table 2 shows the distribution of dry weight between leaves and culm (stem+leaf sheath). Leaf weight ratio (LWR) increased with the increase of N concentration

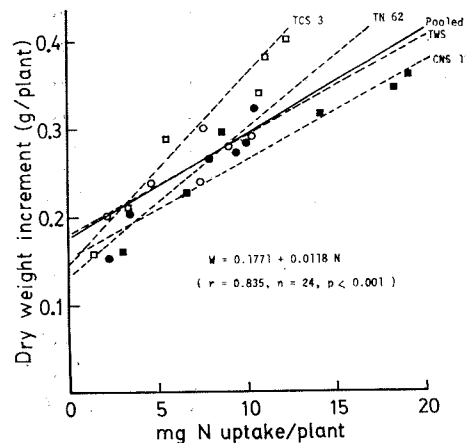


Fig. 2. Relationship between N uptake and dry weight increment. The regression lines were calculated for each rice cultivar grown at six levels of N nutrition. The pooled regression line is also shown (solid line).

**Table 2.** Effect of external nitrogen levels on dry matter distribution (% of total dry weight) (dry weight ratio, DWR) between leaf and stem + leaf sheath

Tissue	Cultivar	Nitrogen levels (ppm N)					
		5	10	20	40	80	120
Leaf	Tainung 62	46.5	47.7	51.0	55.2	51.3	50.2
	Todokiwase	45.6	49.7	49.8	50.3	53.0	52.4
	Chianung sen 11	50.1	57.8	59.1	60.1	62.0	58.5
	Taichung sen 3	48.4	52.6	51.9	55.1	54.2	54.6
Mean	<i>Japonica</i>	46.1	48.7	50.4	52.8	52.2	51.3
	<i>Indica</i>	49.3	55.2	55.5	54.6	58.1	56.6
	Total	47.7	52.0	52.9	53.4	55.2	54.0
Stem + leaf sheath	Tainung 62	53.5	52.3	49.0	44.8	48.7	49.8
	Todokiwase	54.4	50.3	50.2	49.7	47.0	47.6
	Chianung sen 11	49.9	42.2	40.9	39.9	38.0	41.5
	Taichung sen 3	51.6	47.4	48.1	44.9	45.8	45.4
Mean	<i>Japonica</i>	54.0	51.3	49.6	47.2	47.8	48.7
	<i>Indica</i>	50.7	44.8	44.5	46.0	41.9	43.4
	Total	52.3	48.0	47.1	46.5	44.8	46.0

from 46.2% at 5 ppm N to 52.8% at 80 ppm N for *japonica rice*, and 49% to 58% for *indica* cultivars.

Nitrogen treatments also affected the distribution of dry weight between shoots and roots. At low external N level relatively more dry weight accumulated in roots than in shoots and more in roots of CNS 11 than in roots of other three cultivars (Table 3). It seemed that photosynthate was diverted more to roots for root growth in order to increase the nutrient absorption area of roots under severe N limiting conditions. This phenomenon was also found in other plant species (Radin *et al.*, 1978; Reed and Hageman, 1980; Schrader *et al.*, 1972; Turner, 1922; Wallace and Pate, 1967; Theodorides and Pearson, 1981).

Nitrogen contents of leaves and culms increased with the increase of N level (Table 4). No difference in leaf N content was found in plants treated with N above 20 ppm. N content of the culm was much more sensitive to N stress than that of leaves and required higher external N concentration (40 ppm and above) for culm growth. Since the acquisition and distribution of N are closely regulated by the supply of N and plant N demands, the results indicated that N was distributed more to leaves under N stress conditions, implying that leaf is the primary N sink during the growth of rice plants. The average leaf-culm ratio of N content for the four rice cultivars was  $2.85 \pm 0.372$  at 5 ppm N and  $1.77 \pm 0.094$  at 80 ppm N, while

the ratio of leaf N content was  $1.76 \pm 0.136$  (80 ppm/5 ppm) and  $2.95 \pm 0.488$  for the culm. The results showed that only when the available N increased, then culm N content increased.

Nitrogen treatments did not affect phosphorus (P) content (Table 4). P content in all plant organs remained at the same levels in all N treatments. Potassium (K) contents of leaves and culms were also not affected by N treatments, except the culm under serious N stress condition.

*Effect of N Treatment on Rice Growth*

*Relative growth rate:* The relative growth rate (RGR) of the four rice cultivars

**Table 3.** *Effect of external nitrogen levels on root dry weight, shoot dry weight, and root/shoot dry weight ratio of the four rice cultivars*

Cultivar	Nitrogen levels (ppm N)	Root (mg/plant)	Shoot (mg/plant)	Total (mg/plant)	Root/shoot ratio (%)
TN 62	5	64	194	258	33.0
	10	115	526	641	21.8
	20	179	923	1,102	19.3
	40	201	1,221	1,422	16.5
	80	135	1,645	1,780	8.2
	120	400	2,306	2,707	17.0
TWA	5	85	324	409	26.3
	10	143	632	775	22.6
	20	211	884	1,095	23.9
	40	174	1,489	1,663	11.7
	80	67	927	994	7.3
	120	138	1,456	1,594	9.5
CNS 11	5	178	254	432	70.1
	10	267	406	673	65.8
	20	222	687	909	32.4
	40	270	912	1,182	29.7
	80	83	936	1,020	8.9
	120	184	1,195	1,378	15.4
TCS 3	5	80	271	351	29.5
	10	107	418	525	25.6
	20	242	956	1,197	25.2
	40	—	—	—	—
	80	97	1,667	1,765	5.8
	120	88	1,264	1,352	7.0



**Table 4.** Effect of nitrogen levels on N, P, and K contents (% of dry weight) in leaf and stem + leaf sheath

Element	Cultivar	Tissue	Nitrogen levels (ppm N)						
			5	10	20	40	80	120	
N(%)	TN 62	leaf	2.70	2.85	3.90	4.42	4.30	4.62	
		stem+leaf sheath	0.92	0.97	1.99	2.04	2.28	2.44	
	TW	leaf	2.54	2.96	3.48	4.29	3.97	4.53	
		stem+leaf sheath	1.06	1.22	1.62	2.10	2.12	2.34	
	CNS 11	leaf	2.68	3.78	5.44	5.40	5.77	6.08	
		stem+leaf sheath	1.21	1.58	2.32	3.11	3.71	3.46	
	TCS 3	leaf	2.09	2.46	2.57	3.55	3.63	3.24	
		stem+leaf sheath	0.54	1.18	1.10	2.12	2.30	2.18	
	P(%)	TN 62	leaf	0.483	0.394	0.396	0.437	0.417	0.467
			stem+leaf sheath	0.568	0.517	0.522	0.565	0.562	0.565
TW		leaf	0.404	0.409	0.431	0.441	0.376	0.428	
		stem+leaf sheath	0.476	0.479	0.501	0.474	0.474	0.424	
CNS 11		leaf	0.551	0.491	0.560	0.477	0.445	0.406	
		stem+leaf sheath	0.669	0.638	0.554	0.694	0.601	0.552	
TCS 3		leaf	0.426	0.388	0.351	0.333	0.345	0.351	
		stem+leaf sheath	0.398	0.433	0.452	0.392	0.383	0.364	
K(%)		TN 62	leaf	2.99	3.04	3.48	3.55	3.19	3.28
			stem+leaf sheath	4.40	4.89	5.86	5.89	5.91	5.47
	TW	leaf	2.58	3.29	3.22	3.19	2.72	2.94	
		stem+leaf sheath	5.18	5.38	5.62	5.67	5.47	4.40	
	CNS 11	leaf	2.63	3.04	3.84	3.23	2.86	2.31	
		stem+leaf sheath	4.45	5.62	5.82	6.21	5.72	5.18	
	TCS 3	leaf	2.89	3.04	3.19	3.24	2.94	3.09	
		stem+leaf sheath	4.20	5.08	5.87	5.77	4.84	5.67	

showed a saturation growth kinetics as response to N application (Fig. 3). When RGR (dry mass, g/g·d) was decreased 27% (TN 62), 16% (TWS), 31% (CNS 11) and 35% (TCS 3) by N deficiency, the net assimilation rate (NAR, g/cm<sup>2</sup>·d) was decreased 24% (TN 62), 11% (TWS), 19% (CNS 11) and 25% (TCS 3) (Fig. 3) and the relative rate of leaf area increment (m<sup>2</sup>/m<sup>2</sup>·d) was decreased 38% (TN 62), 35% (TWS), 57% (CNS 11) and 54% (TCS 3). The overall growth rate (GR, mg/d) was inhibited 52% (TN 62), 38% (TWS), 56% (CNS 11) and 61% (TCS 3). Thus, N deficiency of equal severity caused considerably greater growth retardation in *indica* rice than in *japonica* rice. The early-mature cultivar TWS seemed relatively less responsive to N stress than the other rice cultivars. The reason for the difference in the response of GR may be that leaf area growth of *indica* rice is affected

to a greater degree by N stress than leaf photosynthesis.

One clear implication of these findings is that *japonica* rice may use N more efficiently (relative to *indica* rice) for dry matter production, particularly when nitrogen is growth limiting.

**Net assimilation rate, leaf area, and growth rate:** The effect of different levels of nitrogen nutrition on net photosynthesis (net assimilation of dry matter) was small. Essentially, the N levels above 10 ppm N had little effect on net assimilation rate (NAR) of dry matter (Fig. 3), and it was affected only at more severe N stress in the four rice cultivars. On the other hand, leaf area growth was much more sensitive to the change of N concentration of the culture solution, particularly the *indica* varieties. Since leaf area is both an expression of size and partial expression of photosynthetic potential and the growth rate can be expressed as  $GR = (NAR) \times (\text{mean leaf area})$ , increase in leaf and/or NAR might promote dry matter production. Therefore, it can be seen in Fig. 3 that GR increased with raising the N level and reached its maximum till at non-limiting level of N supply for rice growth. Nitrogen can influence photosynthesis and growth through affecting leaf area itself (Watson, 1952, 1963; Bouma and Dowling, 1966; Halse *et al.*, 1969) or through changes in photosynthetic rate per unit leaf area (Natr, 1975; Bolton and Brown, 1980). Others have shown that growth responses to changes in N supply were reflected in effects on the NAR as well as on leaf growth (Bouma, 1970; Radin, 1983). The effect of N nutrition on GR may be interpreted as that at lower N the dry matter production of rice is affected by NAR as well as leaf area,

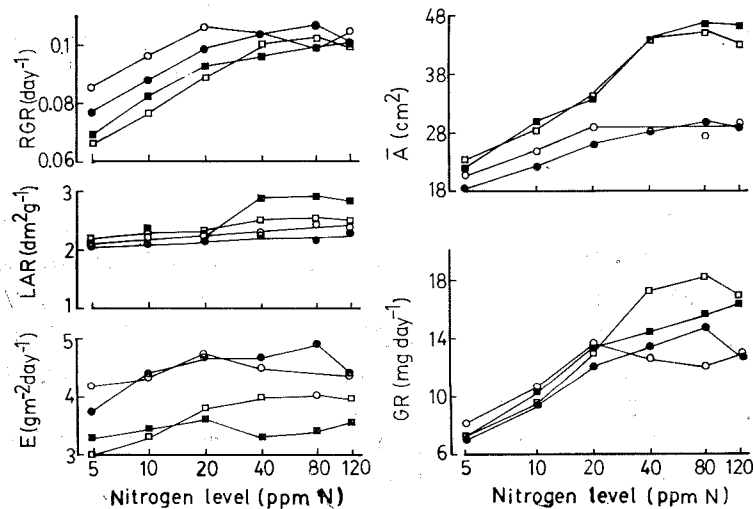


Fig. 3. Effect of nutrient N concentration on relative growth rate (RGR), growth rate (GR), leaf area ratio (LAR), net assimilation rate (E), and mean leaf area ( $\bar{A}$ ).

while the growth of rice plants is mainly controlled by leaf area at higher levels on N nutrition. Imai and Murata (1978) also showed similar results. The interaction of leaf area and NAR, as regulated by nitrogen status of the plant, determined GR and hence dry matter production. It was observed that low N accelerated senescence of older leaves (Table 1). A depletion of N supply may have triggered the mobilization of N from the leaf for the growth of young tissues (Blacklow and Incoll, 1981; Mae *et al.*, 1983; Sinclair and de Wit, 1975). It is therefore no wonder that the photosynthetic CO<sub>2</sub> fixation of the fully-expanded young leaf of rice plants was not affected by the nutrient N concentration in this experiment (Table 5;

**Table 5.** *Effect of external nitrogen levels on leaf area, net CO<sub>2</sub> uptake, chlorophyll and soluble protein content in four rice cultivars*

Each figure was the mean of at least three individual leaves.

Cultivar	Nitrogen levels (ppm N)	Leaf area (cm <sup>2</sup> /leaf)	Net CO <sub>2</sub> uptake		Chlorophyll (μg/cm <sup>2</sup> )	Protein (μg/cm <sup>2</sup> )
			mg/dm <sup>2</sup> ·h	mg/leaf·h		
TN 62	5	26.73	17.36	4.64	10.34	116
	10	41.20	22.56	9.29	10.80	110
	20	39.07	23.30	9.10	15.08	139
	40	40.07	29.00	11.62	21.50	176
	80	40.17	18.74	7.52	24.28	192
	120	43.30	23.43	10.33	24.93	179
TWS	5	34.57	19.13	6.61	9.36	127
	10	35.23	19.24	6.78	8.57	136
	20	39.97	21.44	8.57	13.97	143
	40	49.10	27.34	13.42	18.61	161
	80	51.13	17.07	8.73	17.78	182
	120	43.60	27.68	11.81	18.69	178
CNS 11	5	36.73	38.17	14.01	9.29	130
	10	45.77	34.88	15.60	12.52	130
	20	45.23	29.84	13.54	16.02	131
	40	52.93	23.41	12.39	17.51	154
	80	49.43	27.09	13.39	19.25	186
	120	55.95	32.10	17.96	17.67	132
TCS 3	5	30.10	28.53	8.59	9.51	117
	10	35.17	28.21	9.92	12.03	138
	20	52.77	26.09	13.77	12.52	132
	40	50.20	30.84	15.48	15.23	172
	80	45.00	24.82	11.17	15.07	165
	120	49.41	28.63	14.15	15.70	166

Makino *et al.*, 1984a). However, extra nitrogen may delay the decline of the photosynthetic capacity of leaf (Evans, 1983; Thomas *et al.*, 1978). The differences of whole-plant growth of rice plants between N treatments, which are reflected in their increased GR, then became apparent.

#### Nitrogen Stress

N stress is a quantitative estimate of the intensity of current N deficiency in a plant or crop. It can be expressed as (Greenwood, 1976):

$$S_N = \frac{(\text{Growth rate at maximum N response}) - (\text{growth rate at N deficiency})}{\text{Growth rate at maximum N response}} \times 100$$

The response by rice to N supply was expressed as (1) yield of dry mass (Fig. 1), (2) relative growth rate (RGR) and absolute growth rate (GR) (Fig. 3), (3) N stress based on RGR (Fig. 4), and (4)  $S_{NR}$  vs. leaf nitrogen content (Fig. 4). Results of Fig. 4a closely agreed with that of Fig. 1. All rice cultivars were released of N stress when N supply was 40 ppm N or higher. However, when using leaf N content basis, the results showed variety variation. Cultivar TWS and TCS were much more sensitive to the change of leaf N content and CNS 11 needed higher content of leaf N to recover from N stress. Since the development of leaf area in rice plants was sensitive to nitrogen status, it followed that the rate of increase in leaf area should also reflect accurately the influence of N deficiency on plant growth. Fig. 5 shows a close relation between the increase of leaf area and nitrogen stress. It demonstrated the close relationship between leaf area and the influence of N on growth. At the same level of N supply the *indica* rice were more sensitive to the changes of N supply.

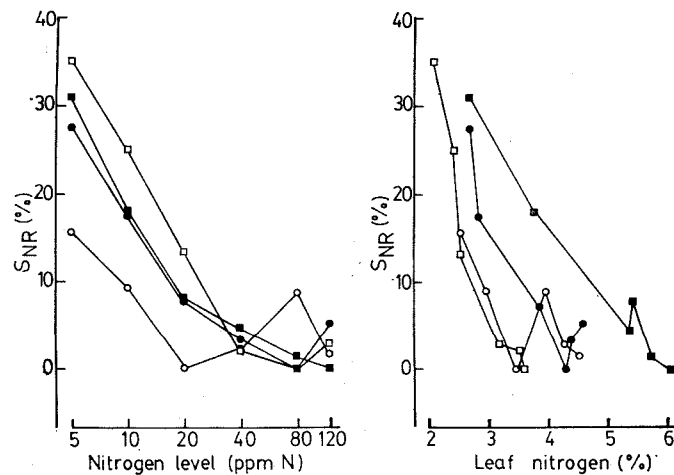


Fig. 4. Relationship between N stress and nutrient N concentration in four rice cultivars.

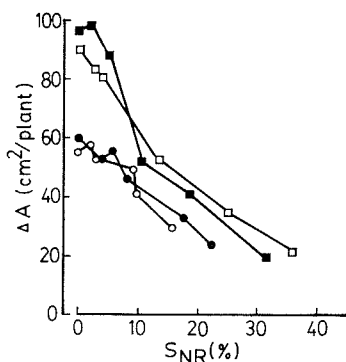


Fig. 5. Relationship between increase in leaf area (A) and nitrogen stress ( $S_{NR}$ ) in four rice cultivars.

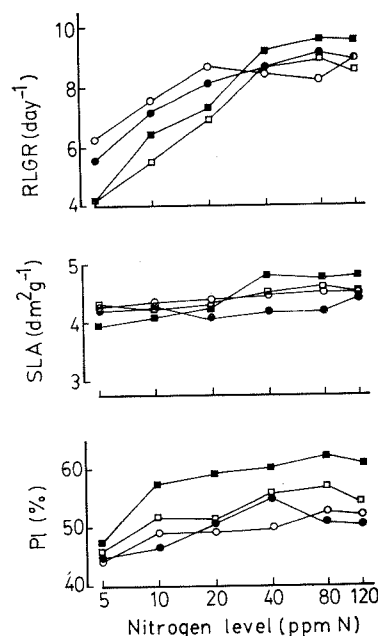


Fig. 6. Effect of nutrient N concentration on relative leaf area growth rate (RLGR), specific leaf area (SLA), and leaf weight partitioning coefficient (Pl).

Analyses of leaf area growth revealed that specific leaf area was less affected by changes in the level of N supply, while leaf weight partitioning coefficient (pl) was affected by N concentration of the culture solution and hence the relative area growth rate (RLGR) (Fig. 6).

#### *Effect on Net CO<sub>2</sub> Uptake, Chlorophyll and Protein Contents*

The fully-expanded youngest leaves of the four rice cultivars were used for the measurements of the net CO<sub>2</sub> exchange, chlorophyll content and buffer soluble protein content. It was surprising to find that difference in N supply did not affect photosynthetic activity on the leaf area basis, except at lower levels of N supply in which chlorophyll and protein contents were also low (Table 5). Similar results were obtained by other workers (Evans, 1983; Makino *et al.*, 1984a). When net CO<sub>2</sub> uptake was expressed on per unit of chlorophyll, the difference in photosynthesis among N treatments largely disappeared (data not shown). It may indicate that the photosynthetic apparatus apparently is not impaired by N stress under the conditions of this studies. It was also noticed that the net CO<sub>2</sub> uptake on a leaf area basis of the *indica* rice CNS 11 was not affected by N treatment, rather a slight increase in net CO<sub>2</sub> uptake per unit of leaf area was shown. Photosynthesis

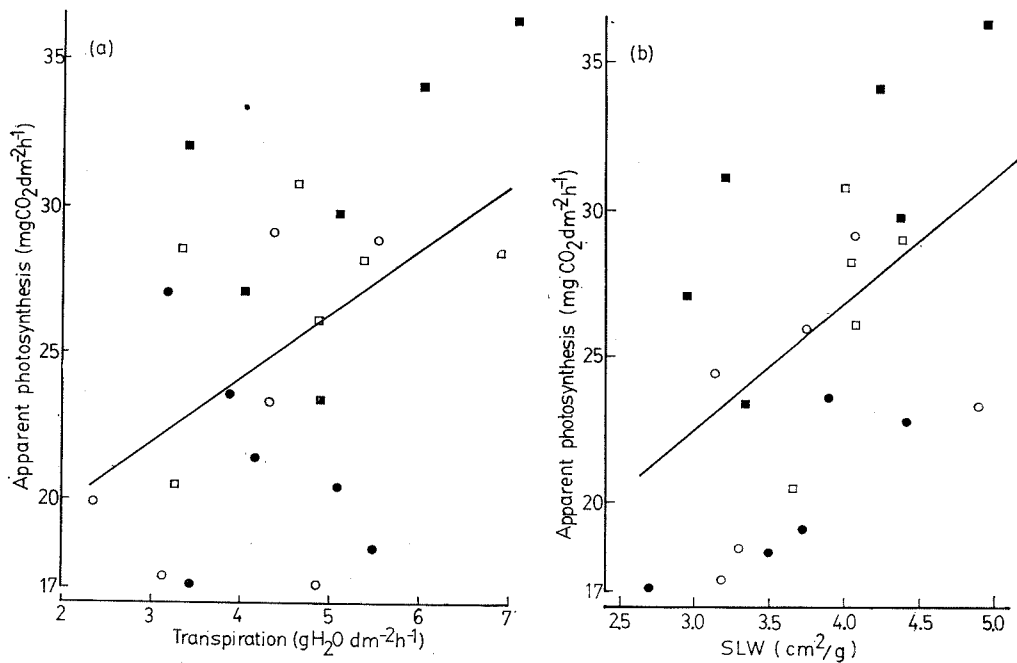


Fig. 7. Relation of net carbon dioxide exchange rate to transpiration (a) and specific leaf weight (b). The regression lines were drawn from the data points of the four rice cultivars grown at six levels of N nutrition. (a)  $P=15.571+2.144 T$  ( $n=24$ ,  $r=0.497$ ); (b)  $P=9.700+4.267 SLW$  ( $n=24$ ,  $r=0.467$ ).

has been shown to be correlated with transpiration (Akita, 1980, Tsuno, 1975), leaf conductance (Makino *et al.*, 1984b) and specific leaf weight (SLW) in the rice plant. Fig. 7 shows that photosynthetic CO<sub>2</sub> uptake was correlated with transpiration and SLW. And particularly, SLW of CNS 11 decreased with the increase of N concentration (Fig. 6). It may account for the compensation of photosynthesis of CNS 11 at low nitrogen level. Correlations have also been established between SLW and photosynthesis in other plant species (Dornhoff and Shibles, 1976; Hart *et al.*, 1978; Hunt and Halligan, 1981; Pearce *et al.*, 1969).

#### *Effect of Nitrogen on Enzyme Activity*

Figure 8 (upper) shows the influences of N supply on the ribulose-1,5-bisphosphate carboxylase (RuBPCase) activity and the ratio of RuBP oxygenase to RuBPCase (RuBP O/C ratio). The RuBPCase may be expressed in relation to unit leaf area, soluble leaf protein and/or chlorophyll content. There was no effect of N treatment on the RuBPCase activity if the activity was expressed per unit protein and/or chlorophyll (data not shown), while the activity per unit leaf area was affected by N level. It can be seen that the RuBPCase decreased in low N

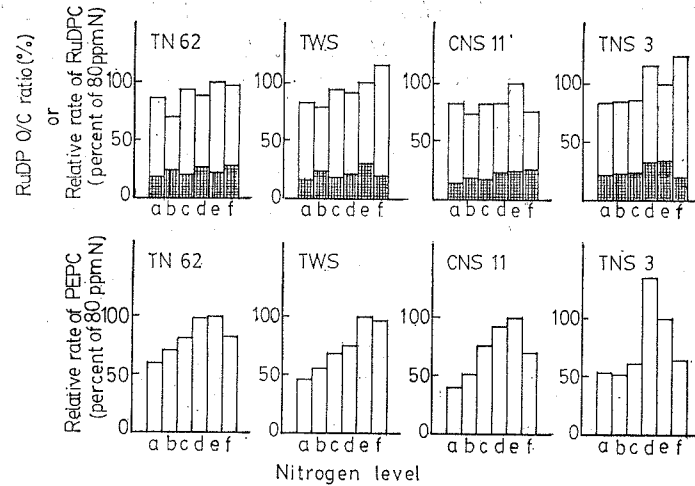


Fig. 8. Effect of nutrient N concentration on RuBPCase activity (unshaded), RuBP O/C ratio (shaded), and PEPCase activity. RuBPCase and PEPCase activities are expressed as the percentage of the rate of 80 ppm N treatment. a, b, c, d, e, and f represent the nutrient N concentration of 5, 10, 20, 40, 80, and 120 ppm N, respectively.

level, but the decrease in the enzyme activity under N stress was not very great. The difference was about 10% to 15% between low and elevated N levels.

Since RuBPCase protein or Fraction I protein accounted for half or more of the soluble leaf protein in the rice leaf (Mae *et al.*, 1983) and the soluble leaf protein increased with the increase of N levels (Table 5), it is not surprising that the NAR was affected by low N, but only at more severe N stress (Fig. 3; Table 5). Makino *et al.* (1983) showed that RuBPCase activity in a rice leaf was highly correlated with the net rate of photosynthesis. Nitrogen limitation was found to reduce the RuBPCase in rice (Makino *et al.*, 1983, 1984) and other plant species (Evans, 1983; Fritsch and Jung, 1984; Thomas *et al.*, 1978). On the other hand, the specific activity of RuBPCase was commonly found maintained fairly constant in several plant species (Evans, 1983; Fritsch and Jung, 1984; Makino *et al.*, 1983; Peoples *et al.*, 1980; Thomas *et al.*, 1978). It may indicate that in the rice plant and other plant species RuBPCase activity is regulated by its synthesis and degradation and the synthesis of RuBPCase in a leaf is controlled by the amounts of N influx into the leaf. Evans (1983) also indicated RuBPCase activity was approximately proportional to leaf N content and rate of CO<sub>2</sub> assimilation was uniquely related to total leaf nitrogen irrespective of nutrient treatment, season and leaf age. The results in the present experiment showed that CO<sub>2</sub> exchange rate of a single fully-expanded leaf was related to leaf N content, but irrespective of N treatment (Makino *et al.*, 1984a).

Figure 8 also shows the ratio of RuBP oxygenase to RuBPCase. There was no significant effect of N on the RuBP O/C ratio. The same was reported by other workers (Fritsch and Jung, 1984; Hall and Keys, 1983; Kabaki *et al.*, 1979; Peoples *et al.*, 1980).

The activity of phosphoenolpyruvate carboxylase (PEPCase) was more sensitive to the changes of N supply. PEPCase activity was inhibited by about 50% in low N supply (Fig. 8, lower). The role of PEPCase in the rice leaf is not clear. However, PEPCase activity in an expanding leaf declined sharply before RuBPCase activity reached its maximum (data not shown). Kundu *et al.* (1978) proposed that the main function of leaf PEPCase in  $C_3$  plants such as the rice plant appears may be the supply of dicarboxylic acids, but Hedley *et al.* (1975) suggested a retrapping  $CO_2$  mechanism of PEPCase.

Increase in the external N supply resulted in increasing in leaf blade *in vitro* nitrate reductase (NRA) and nitrite reductase (NiRA) activities (Fig. 9). The effects of N stress on NRA and NiRA were more pronounced than on RuBPCase and PEPCase. NRA and NiRA at the elevated N were 8 to 10 and 3 to 4 times greater than those at severe N stress, respectively. NRA was more sensitive to the changes in N supply than NiRA in both *japonica* and *indica* rice cultivars.

Oji and Izawa (1970) showed that in nitrate-supplied rice plants, most of the absorbed nitrate was transported to the shoot for reduction. Shen (1969) found NR even in 3.5-day-old rice seedlings and showed rice seedlings could assimilate nitrate from the beginning of germination. In the present experiment,  $NH_4NO_3$  was used as the sole N source to raise the rice seedlings. Shen (1969) and Sasakawa and Yamamoto (1978) indicated that nitrate uptake was suppressed by the presence of ammonium. Therefore, our results refer that the increase in the N reduction enzyme activities reflects an increase in the amount of the enzymes and N stress inhibits

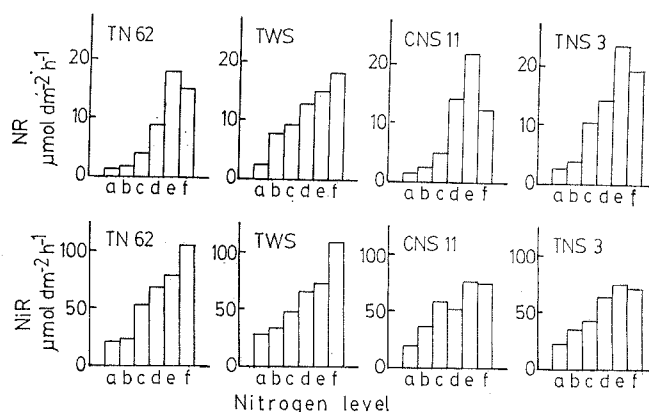


Fig. 9. Effect of nutrient N concentration on nitrate reductase (NR) and nitrite reductase (NiR) activities in four rice cultivars.



the N influx (Reed and Hageman, 1980). Since the activities of NRA and NiRA decreased at N stress as expressed in relation to both unit leaf area and soluble leaf protein basis and the specific activity of RuBPCase (per unit protein) remained constant irrespective of N treatment, it may imply that the synthesis of RuBPCase protein was the prime N metabolic sink in the rice leaf.

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## 氮素對水稻生長及光合作用的影響

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四個水稻品種的稻苗在四葉齡時以 5~120 ppm N 等六種濃度的硝酸銨處理三個星期，然後取樣調查植株的乾物質、葉面積、葉綠素及可溶性蛋白質含量，以及酵素活性，以期瞭解氮素吸收及代謝對水稻的生長及光合作用的影響。稻株乾物重的生長對氮素濃度呈飽和型生長動力，但此曲線因水稻品種而略有不同。水稻乾物重的增加量與植株氮素的吸收量呈正相關。生長分析顯示氮素主要影響葉面積的生長，僅在較低的氮素濃度下淨同化作用受到抑制。淨光合作用  $\text{CO}_2$  吸收與蒸散速度及比葉重 (specific leaf weight) 呈正相關。葉片的葉綠素含量和可溶性蛋白質含量與全氮素量呈正相關。葉片的光合作用速率在 10~120 ppm N 的氮素濃度處理間無顯着差異，但植株生長量隨氮素濃度的提高而增加，粳稻在 40 ppm N，秈稻在 80 ppm N 達最大生長量。雙磷酸核酮糖加羧酶 (RuBPCase)，磷酸丙酮酸加羧酶 (PEPCase)，硝酸及亞硝酸還原酶的活性均隨氮素處理濃度之增加而提高，尤以硝酸還原酵素對 N 的感應最為明顯。但 RuBPCase 的比活性和 RuBP O/C 比却不受氮素處理的影響。本試驗的結果提示水稻的生長受到氮素吸收及代謝的控制，而葉面積的生長為主要的限制機制。