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Interaction of Nitrogen Supply and Soil Water Stress on Photosynthesis and Transpiration in Rice*

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Abstract : The purpose of this study is to determine if there is an interaction of nitrogen nutrition and water stress on apparent photosynthesis (APS), transpiration (Tr), and water-use efficiency in rice (*Oryza sativa* L.). The potted plants, which were cultured in high and low nitrogen levels, were exposed to the water stress condition, and the gas exchange rates were measured. A significant interaction of nitrogen supply and soil water stress on APS and Tr occurred, with high nitrogen plants being more affected by water stress, in respects of APS and Tr, compared with low nitrogen plants. This high responsiveness of APS and Tr in high nitrogen plants was due to stomatal sensitivity to water stress. In addition, high nitrogen plants maintained better photosynthetic machinery under water stress condition. As the results, it was found that the characteristics of greater stomatal sensitivity and better photosynthetic machinery in high nitrogen plants, led to their higher water-use efficiency.
Key words : Nitrogen supply, *Oryza sativa* L., Photosynthesis, Rice, Stomata, Water stress, Water use efficiency.

イネの光合成と蒸散速度におよぼす窒素施肥と土壌水分欠乏の相互作用：オトー　アーネスト・石井龍一・玖村敦彦（東京大学農学部）

要　旨：本研究は，イネのみかけの光合成速度，蒸散速度，水利用効率に対して，窒素施用量と土壌水分欠乏処理との間に相互作用が見られるかどうかを明らかにしようとしたものである．すなわち光合成速度，蒸散速度，水利用効率に対する土壌水分欠乏の影響が，窒素の施用量によって変化するかどうかを明らかにしようとしたものである．実験には1/2,000 a ワグナーポットを使用し，3.2 g，0.4 g の2段階の窒素条件下で植物を生育させた．7葉期に達した植物に土壌水分欠乏処理を施し，それらのガス交換速度を測定した．その結果，有意な相互作用が認められ，多窒素施用区の植物体は，光合成，蒸散速度のいずれにおいても少窒素施用区の植物体より土壌水分欠乏の影響を大きくうけていた．特にその影響は蒸散速度において大きかった．このことから，多窒素施用区の植物体では，その気孔が水ストレスに対して，より敏感に反応することが考えられた．同時に，多窒素施用区の植物体では葉肉細胞内の光合成系の活性が高く保たれており，少窒素施用区の植物体に比較して水ストレス条件下でも大きい光合成速度を有していた．このように多窒素施用区の植物体は，水ストレス条件下でも光合成系の活性を高く維持させるとともに，水ストレス条件に対して気孔を敏感に反応させることによって水利用効率を高く保っていることが明らかとなった．
キーワード：イネ，*Oryza sativa* L.，気孔，光合成，窒素施肥，水ストレス，水利用効率．

Drought tolerance and nitrogen responsiveness are recognized as desirable characteristics of crop plants, in breeding for high yielding cultivars in semi-arid regions. Hence, the interactive effects of nitrogen nutrition and water stress on plant growth have received attention from several workers. Viets[23] had earlier suggested that the growth response of crop plants to irrigation depends on the availability of nitrogen in the soil. Recent evidences also suggest that the internal water relations of crop plants during water stress, depends on the supply of nitrogen[7,9-11,14-20,22,25]. However, there is a dearth of information on the combined effects of nitrogen nutrition and water stress on such a vital physiological process as photosynthesis, though in rice plants the independent effect of either nitrogen supply or water stress on single leaf photosynthesis is well documented[2,12,21,26].

Hence, the objective of this work was to examine the combined effects of nitrogen supply and soil water stress on photosynthesis, stomatal conductance, and water-use efficiency of rice plants.

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Abbreviations : LWP, leaf water potential ; APS, apparent photosynthesis ; Tr, transpiration ; gs, stomatal conductance ; gm, mesophyll conductance ; WUE, water-use efficiency ; ABA, abscisic acid

Materials and Methods

Plant materials and culture:

Todorokiwase, Tsukubahatamochi, IRAT 109, and Kinangdang Puti, representative of improved lowland and upland japonica, and improved and local indica cultivars, respectively, were used. The seeds were sown and plants raised by the procedures described previously[12]. When the plants reached the 4th leaf stage, they were transplanted to 1/2,000 a Wagner pots. To make sure that plants experience the same stress conditions, seedlings of all the cultivars were planted in the same pot. To minimize the differences in the development of soil water stress resulting from differences in leaf area enlargement between the nitrogen treatments, seedlings were transplanted at the rate of two seedlings per cultivar (8 plants in total) for high nitrogen pots and three per cultivar (12 plants in total) for low nitrogen pots. Nitrogen was applied at the rate of 0.4 g N per pot by the form of compound fertilizer (10-16-18), with supplimentary addition of ammonium sulfate to reach 3.2 g N for high nitrogen. The potted plants were initially grown in the field before being transferred to a glasshouse maintained under day/night temperatures of 25/22°C and a relative humidity of 60%. When plants reached the 7th leaf stage, the water stress treatment was started. For plants to be stressed, watering was stopped and they were allowed to deplete soil water till a resistance of 400 ohms attained, at which point leaf water potential and gas exchange rate were determined. High and low nitrogen plants took 7 and 14 days, respectively, to reach the same soil resistance, mainly due to differences in leaf area. Control plants were kept under flooded conditions. The plants were moved to a growth cabinet, 24 h prior to gas exchange measurements in order to acclimatize the whole plant to the conditions of the measuring environment.

Determination of leaf water potential:

Pre-dawn leaf water potential was determined just before lights were turned on in the growth cabinet. The fully expanded 8th leaf of each cultivar was used for the determination by the pressure chamber method.

Determination of gas exchange rate:

Apparent photosynthesis and transpiration rates per unit leaf area were measured on the fully expanded 8th leaf with a semi-closed measuring system (Koito KMC-1000). The plants were transferred to the cabinet 48 h prior to gas exchange measurement. The measurement was conducted in the growth cabinet with a leaf enclosed in the leaf chamber, to avoid a large difference of the environmental conditions between the measured leaf and the whole plant body. Lights in the cabinet were turned on at 6 : 30 and off at 18 : 30 h. Gas exchange measurenents were carried out between 10 : 00 and 14 : 00 h. The leaf chamber was maintained at 25°C and 70% of air temperature and relative humidity, respectively. Incident light intensity was 800 μmol quanta/m^2/s, and CO_2 concentration of the introduced air was maintained at 350 $\mu l/l$ by mixing CO_2 depleted air and 5% CO_2 balanced with nitrogen gas.

Results

Leaf water potential (LWP) :

As shown in Table 1, mean pre-dawn LWP for control plants was around −0.1 MPa, and those for the high and low nitrogen stressed plants were around −0.31 and −0.28 MPa, respectively. This implies that under mild water stress, nitrogen nutrition did not have any significant effect on the ability of plants to regain turgor.

Photosynthesis (APS) :

As shown in Table 2, the response of APS to water stress was affected by the supply of nitrogen. A significant interaction between nitrogen supply and water stress was obtained (1% level of significance). Whereas in the high nitrogen plants, water stress caused a 17% decrease in APS on average, the effect was negligible in the low nitrogen plants. It was, however, observed that APS in high nitrogen plants was higher under both control and stressed conditions, than that in low nitrogen plants. It can, therefore, be inferred that high nitrogen plants maintain larger stomatal sensitivity to water stress, with better performance of photosynthesis compared with low nitrogen plants.

Differences in the response of APS to water stress, were not found consistently between indica and japonica cultivars, and lowland and upland cultivars, although IRAT 109, an improved indica cultivar, was the most tolerant, and Todorokiwase, an improved japonica

Table 1. The effect of nitrogen supply and water stress on pre-dawn leaf water potential (LWP).

Cultivar	Pre-dawn LWP (MPa)			
	High-N-plant		Low-N-plant	
	Control	Stressed	Control	Stressed
IRAT 109	−0.11	−0.31	−0.11	−0.29
Kinangdang Puti	−0.07	−0.22	−0.11	−0.27
Tsukubahatamochi	−0.12	−0.35	−0.10	−0.23
Todorokiwase	−0.11	−0.37	−0.11	−0.33
Mean	−0.10	−0.31	−0.11	−0.28

Values are means of 4 replications.

cultivar, was the least tolerant to water stress condition.

Transpiration (Tr) :

The response of Tr to nitrogen and water stress followed a trend similar to APS. Table 2 shows that high nitrogen plants maintained higher rates of Tr than the low nitrogen plants under both control and stressed conditions. Also, with regard to the relative rate of decrease by water stress, a trend similar to APS was observed. A greater rate of decrease (35%) in high nitrogen plants compared with low nitrogen plants (12%) was apparent. This shows that high nitrogen plants were more sensitive than low nitrogen plants in their response to water stress.

The consistent cultivar difference was not observed for Tr, as in APS.

CO_2 diffusion conductances :

Table 3 shows the response of stomatal (gs) and mesophyll (gm) CO_2 diffusion conductances to water stress as calculated from the data of APS and Tr. Whereas water stress in high nitrogen plants caused a decrease in gs by 40%, only a 15% decrease was seen in the low nitrogen plants. The data regarding the behavior of stomata suggest a greater sensitivity of high nitrogen plants to water stress compared with low nitrogen plants.

With regard to gm, on the other hand, the nitrogen-water stress interaction was not significant ; the decrease of gm due to water stress was not significantly different between high and low nitrogen plants. However, gm values in high nitrogen plants were almost twice those of the low nitrogen plants. This

Table 2. The effect of nitrogen supply and water stress on apparent photosynthesis (APS) and transpiration (Tr).

Nitrogen supply and Cultivar	APS ($mgCO_2/dm^2/h$) Control,	Stressed	Tr ($gH_2O/dm^2/h$) Control,	Stressed
High-N				
IRAT 109	34.5	31.5(− 9)	3.26	2.36(−27)
Kinangdang Puti	33.0	26.2(−21)	3.00	1.89(−39)
Tsukubahatamochi	33.6	26.9(−20)	3.27	2.33(−29)
Todorokiwase	31.8	25.4(−20)	2.77	1.50(−46)
Mean	33.2	27.5(−17)	3.08	2.03(−35)
Low-N				
IRAT 109	17.3	17.4(1)	2.09	2.00(− 4)
Kinangdang Puti	12.2	13.7(12)	1.92	1.64(−15)
Tsukubahatamochi	13.9	15.4(11)	1.63	1.51(− 7)
Todorokiwase	18.3	16.0(−13)	1.84	1.46(−21)
Mean	15.4	15.6(1)	1.87	1.65(−12)
F values[1)]	27.5**		41.0**	

Values are means of 4 replications.
Figures in parenthesis are percentage change relative to control.
1), F values for the interaction between nitrogen and soil water levels.
**1% level of significance.

Table 3. The effect of nitrogen supply and water stress on stomatal (gs), and mesophyll conductance (gm).

Nitrogen supply and Cultivar	gs (cm/s) Control	Stressed	gm (cm/s) Control	Stressed
High-N				
IRAT 109	0.60	0.44(−27)	0.22	0.21(− 5)
Kinangdang Puti	0.58	0.30(−48)	0.21	0.20(− 5)
Tsukubahatamochi	0.63	0.40(−47)	0.20	0.18(−10)
Todorokiwase	0.50	0.24(−52)	0.20	0.22(−10)
Mean	0.58	0.35(−40)	0.21	0.20(− 5)
Low-N				
IRAT 109	0.36	0.35(− 1)	0.10	0.10(0)
Kinangdang Puti	0.35	0.27(−23)	0.10	0.08(−20)
Tsukubahatamochi	0.27	0.25(− 7)	0.09	0.09(0)
Todorokiwase	0.31	0.23(−26)	0.11	0.09(−10)
Mean	0.32	0.28(−15)	0.10	0.09(−10)
F values[1)]	44.5**		0.61ns	

Values are means of 4 replications.
Figures in parenthesis are percentage change relative to control.
1), F values for the interaction between nitrogen and soil water levels.
**1% level of significance. ns, not significant.

implies that a high supply of nitrogen protects the photosynthetic machinery under water stress conditions.

Discussion

A significant interaction between nitrogen supply and water stress was observed in the rice plants studied. Our results show that the response of APS to water stress is dependent on the supply of nitrogen, with a high supply of nitrogen making plants more sensitive in their response to water stress (Table 2). This finding is similar to that observed in bean[19,20], coffee[22], and wheat[9,10], but contradictory to that reported in cotton, where stomatal behaviour of low nitrogen plants was found to be more sensitive than in high nitrogen plants[15−18].

The observation of an interaction between nitrogen supply and water stress on photosynthesis, could have some implications in the breeding of plants with high photosynthetic capability under conditions of mild water stress, as is often experienced by crop plants during the growing season. In rice, single leaf photosynthesis is an important growth determining factor, and under well-watered conditions, a high and positive correlation exists between nitrogen supply and APS[12,21]. Therefore, the observation that high nitrogen plants maintained high APS not only under ample water conditions, but also under water stress conditions, is interesting from the view point of breeding for high nitrogen responsive cultivars for the arid and semi-arid regions. As shown in Table 2, though water stress caused a

Table 4. The effect of nitrogen supply and water stress on water-use effeciency (WUE).

Cultivar	WUE ($mgCO_2/gH_2O$). High-N-plant Control	Stressed	Low-N-plant Control	Stressed
IRAT 109	10.6	13.1(24)	8.7	9.1(5)
Kinangdang Puti	11.2	14.1(26)	6.7	8.7(30)
Tsukubahatamochi	10.4	12.1(16)	10.0	10.3(3)
Todorokiwase	11.9	17.0(44)	10.1	11.5(14)
Mean	11.0	14.1(28)	8.9	9.9(13)

Values are calculated from data of APS and Tr (Table 2). Figures in parenthesis are percentage change relative to control.

greater rate of decrease in APS in high nitrogen than in low nitrogen plants, the former still maintained higher APS than the latter. The activity of ribulose-1, 5-bisphosphate carboxylase has been reported to be greater for high nitrogen as compared to low nitrogen plants[8]. It may, therefore, be inferred that high nitrogen supply, not only protects the photosynthetic machinery from severe damage, but also make plants more sensitive in their response to water stress through stomatal behaviour (Table 3).

The leaf conductance data show that a high supply of nitrogen results in more open stomata that respond better to decreases of water potential. The high stomatal sensitivity of the high nitrogen plants might be related to their high photosynthetic activity, since a coupling of photosynthesis to stomatal closure has been reported[24]. However, the possible involvement of abscisic acid (ABA) cannot be ruled out, since stomatal response is said to be affected by ABA accumulation[17]. Also, the minimal response of the low nitrogen plants may be a reflection of xeromorphic adaptation[6,20].

A result of the differences in photosynthetic activity and stomatal sensitivity between low and high nitrogen plants is reflected in the physiological efficiency of water use. According to Fisher and Turner[3], in the semi-arid and arid regions the efficiency of carbon gain per unit water loss, water-use efficiency (WUE), is an important indicator of plant performance. WUE calculated from the ratio of APS and Tr shows that a high supply of nitrogen increases the WUE of plants, especially, under stressed conditions (Table 4).

In conclusion, a high supply of nitrogen results in higher photosynthetic activity and makes stomata more sensitive to water stress. This results in a better WUE in high nitrogen plants as compared with the low nitrogen plants.

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