

NITROGEN INDUCED CHANGES IN RICE PLANTS: EFFECTS ON HOST-INSECT INTERACTIONS

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ABSTRACT: Effects of nitrogen (N) on growth, biomass, succulence and chemical composition of rice plants and on the host-insect interaction were determined through culture solution at 29°C/21°C day/night temperature and 70% RH at International Rice Research Institute, Philippines. Rice plants grown at low level of nitrogen (5 mg/l) in culture solution were with narrow, short and erect leaves and their height was 40% less than the plants grown in standard culture solution (40 mg N/l). Number of tillers per hill increased significantly with corresponding increase in the application of N. Excessive N application (200 mg/l) significantly reduced the root length and biomass than the plants grown in standard culture solution. Root to shoot ratio increased with corresponding increase in the application of N. Nitrogen and carbon contents in the leaf sheath of rice plants increased with corresponding increase of N. However, the quantity of silica and zinc and C-N ratio decreased with increase in the application of N. Increase in N induced succulence in rice plants which makes them more prone to insect pests. Changes in the level of N in the culture solution for raising rice plants profoundly affected the host-insect interactions. Intake and assimilation of food, growth and development, adult longevity, fecundity and population increase of whitebacked planthopper, *Sogatella furcifera* was significantly higher at high level of N than at low level of N.

Key Words: Rice; Nitrogen; Root; Shoot; Growth; Insect Pests; Chemical Composition; Succulence; Philippines.

INTRODUCTION

Plant nutrients are the elements or simple inorganic compounds, indispensable for their growth which are not synthesized by the plants during the normal metabolic processes. All the 16 essential elements must be present in optimum amounts and forms usable by rice plants (De Datta, 1981). Innumerable interactions of one element with others are known (Hewitt, 1963; Hiatt and Leggett, 1974; Olsen, 1972; Smith, 1962) and these could be of major significance (Clark, 1982).

Nitrogen is essential for all living organisms. The synthesis of cellular proteins, amino acids, nucleic acids, purine and pyrimidine nucleotide are dependent upon N. It is the most abundant mineral element in plant tissues which is derived from the soil. However, excess N may cause significant biochemical changes in plants and may lead to nutritional imbalances (Mills and Jones, 1979). Generally it is

needed in most rice soils. Rice plants require a large amount of N at the early and mid tillering stages to maximize the number of panicles (De Datta, 1981).

Little information was available on the effects of deficiency and excess amount of N on the shoot and root growth, biomass production and chemical composition of rice plants. Therefore some studies were carried out on these aspects.

MATERIALS AND METHODS

Different precise levels of N were maintained through culture solution, which was prepared following the procedure of Yoshida et al. (1976) in demineralized water.

Preparation of Culture Solution

Four litre culture solution with 5 (low), 40 (standard) and 200 mg N/l (high) were prepared by adding 0.63, 5 and 25 ml of ammonium nitrate stock solution, respectively. However, 5 ml of other stock solu-

tions were used in each case.

Growing of Rice Plants

Seed of Taichung Native I (TNI susceptible to insect) and IR2035-117-3 (resistant to insect) rice cultivars were surface sterilized with 0.1% mercuric chloride solution for 2 minutes and washed with demineralized water to remove mercuric chloride. Seeds were soaked for 24 hours and spread on nylon net stretched on styrofoam frame floating in a plastic tray having 50% culture solution of pH 5. The tray was placed in a glass house under natural daylight, day/night temperature 29°C/21°C and 70% RH. The seedlings were transplanted in porcelain pots 14 days after soaking. The experiment was laid out in RBD with four replications. Each treatment contained ten rice plants. The solution was changed at weekly intervals. The pH of the solution was adjusted to 5.5. Plant height and root length were measured and tillers were counted. Forty days after transplanting, the rice plants were removed and washed with demineralized water. Roots and shoots were placed in marked paper bags and dried in a Hurricane-oven at 80°C for 72 h and weighed separately. Fresh weight of shoot and root was also recorded. Root to shoot ratios were determined and succulence ratio of rice plants was also calculated.

Chemical Analysis of Plants

Forty days old rice plants grown at different levels of N were cut and dried at 80°C for 72 h and analyzed for N, P, K, Mg, Ca, Si, Zn, Fe, total free sugars, and soluble proteins. For analysis of total N, 50 mg of ground sample was digested in a micro Kjeldahl flask with 2 ml of concentrated sulphuric acid and 1 g catalyst mixture (Se: K₂SO₄, 20:100 g) until clear and cooled and made up to 20 ml with deionized distilled water. Nitrogen was determined colorimetrically as indo-phenol blue on a Technicon Autoanalyzer II (TAA). For analysis of P, Fe, and Si 1 g of sample was digested with a 10 ml mixture of HClO₄, HNO₃ and H₂SO₄ with ratio 300, 750, 150 ml on a hot plate at 500°C until a gelatinous white residue remained to which deionized wa-

ter was added after cooling. The mixture was filtered through Whatman No.42 to a 50 ml volumetric flask. The residue on the filter paper was washed twice with 0.1M HCl and the washing were added to the filtrate. The filtrate was made up to the volume with 0.1M HCl and shaken well. Phosphorus was determined colorimetrically using acid molybdate method on Technicon Autoanalyzer. Iron was analyzed using Atomic Absorption Spectrometer (AAS) (Perkin Elmer, model # 2380). The residue on the filter paper was burnt at 500 °C for 4 to 5 h for gravimetric determination of crude Si. For analyses of K, Ca, Mg and Zn, 1 g of plant sample was soaked for 24 h in 25 ml 1M HCl and filtered through Whatman No.43. Potassium, Ca, Mg, and Zn were analyzed in the filtrate using AAS. For estimation of soluble proteins and free sugar, plants were analyzed on dry weight basis (Lowry et al., 1951 and Yoshida et al., 1976).

Insect Responses

Whitebacked planthopper, *Sogatella furcifera* reared on potted TNI plants for several generations in a greenhouse was used as test insect. The insect responses included orientation, intake and assimilation of food, growth and development, adult longevity, fecundity, and population increase.

Oriental Response

The orientational response of brachypterous *S. furcifera* females was tested using cylindrical glass chambers (each 70-mm long, 130mm diameter) having nylon-net walls at both ends (Saxena et al., 1974). Plants that were raised in culture solution at low level of N (5ppm) were placed singly with roots immersed in the culture solution in 8 vials at one end of the chamber, while control plants, raised at 40 ppm N in culture solution were placed at the opposite end of the chamber. Ten newly emerged females (starved for 2 hours but water satiated) were introduced into the middle of the chamber using a mouth aspirator. The females could respond to the plants but could not come in contact with them because they were outside the nylon net walls. Fe-

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males alighting on the end-walls of the chamber facing the plants were counted at 5min. intervals for one hour to observe their orientational response. Each treatment was replicated eight times. Likewise another set of experiment was conducted with control rice plants (40 ppm N in culture solution) and the plants raised at high level of N (200 ppm). Chi square tests were performed to determine whether observed ratios of females attracted to N-deficient and control plants deviated significantly from the no preference ratio of 1:1.

Intake and Assimilation of Food

Newly emerged brachypterous females (starved for 3 hours but water satiated) were weighed in batches of three and enclosed in air-tight parafilm sachet (50 by 50 mm), through each of which passed the leaf sheath of rice plants grown for 35-days in culture solution at 5, 40 or 200 ppm N. After 24 hours, each batch of females and their excreta were weighed. To assess the loss in insect body weight due to catabolism, a control was similarly established, in which the females were given access to a moist cottonswab to prevent desiccation. The amount of food ingested and assimilated was calculated as described by (Saxena and Pathak, 1977).

Growth and Development

Plants grown at different levels of N in culture solution for 35 days were placed singly in plastic pots (140 mm deep, 120 mm diameter) containing respective concentration of N in the culture solution. Roots of each plant were kept immersed in the solution while tillers emerged through a median hole (15 mm diameter) in a circular styrofoam lid (20 mm thick, 122 mm diameter) on the pot. A mylar-film sleeve (360 mm high 122.5 mm diameter) was slipped over the styrofoam lid upto the base of the pot. A cylindrical mylar film cage (1000 mm high, 120 mm diameter) with a nylon-mesh top and side windows enclosed each plant and snugly rested on the styrofoam lid within the collar of the mylar-film sleeve. All plants were infested with first instar nymphs of *S. furcifera* in batches of 10 each.

The infested plants were arranged in a randomized complete block design. There were four replicates for each treatment. The pH of the culture solution was adjusted daily to 5.5 and the culture solution was changed at weekly intervals. Growth was measured by the number of nymphs that became adults and time taken to reach the adult stage. The insect growth index was calculated as the ratio of percentage nymphs becoming adults to the mean growth period in days (Saxena et al. 1974).

Adult Longevity and Fecundity

Plants grown in culture solution at different levels of N (5, 40 and 200 ppm N) in plastic pots were infested with 10 pairs of newly emerged females (brachypterous) and males and covered with mylar-film cages, as described above. Insect mortality was registered daily until all individuals died. The total number of nymphs emerged on each plant was recorded and represented the number of viable eggs laid by the females. At the end of the nymphal emergence, unhatched eggs were also counted by dissecting leaf sheaths under a binocular microscope. The total number of nymphs emerged and the number of unhatched eggs represented the fecundity of *S. furcifera*.

Population Development

Plants grown in the culture solution at three different levels of N (5, 40, 200 ppm) were shifted @ 4 plants/pot (180 mm deep, 160 mm diameter) and infested with five pairs of newly emerged females (brachypterous) and males using mylar cages (1000 mm high, 170 mm diameter), as described above. Insects, both nymphs and adults, were counted 40 days after infestation.

Data for tests on food intake and assimilation, growth, adult longevity and fecundity, and population increase were subjected to analysis of variance and the means were compared using Duncan's (1951) multiple range test at the $P < 0.05$ and the least significant difference test at $P < 0.05$ and 0.01 levels (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Plant Growth

Rice plants grown at low level of N (5ppm) in culture solution were with narrow, short and erect leaves which became yellowish green with age. However, old leaves were of light straw colour. Height of rice plants grown at low level of N was significantly less than the plants grown in standard (40ppm) culture solution (Table 1). Decrease in plant height grown at low level of N than the plants in standard culture solution was more with increase in age in both the test rice cultivars. It was significantly higher at high level of N (200ppm) than the plants grown in standard culture solution (40ppm).

Tillering is a characteristics of the rice plant, particularly that of transplanted rice. Each node of a shoot has a tiller primordium. Whether the primordium develops into a tiller depends on the nutritional status of the plant (Yoshida 1981). During present investigation, number of tillers per plant were significantly higher in the plants of both cultivars grown in standard culture solution (40ppm) than the plants grown at low level of N (5 ppm) even at 10 DAT. However, the difference in the number of tillers among the plants grown at different levels

of N became more pronounced with increase in the plant age. Number of tillers increased significantly with corresponding increase in the application of N at 20 and 40 DAT. The results of present investigation agree with Yoshida (1981), who reported 50% less number of tillers at low level of N in culture solution. Increase in the level of N to 200ppm increased 40% and 32% tillers in TNI and IR2035-117-3, respectively than the plants grown in standard culture solution. Our results also agree with Son et al. (1988) who stated that tiller numbers were higher at high N treatment.

At 10 DAT root, length of TNI plants grown at different levels of N was same but that of IR2035-117-3, it was significantly less at high level of N. However at 20 and 40 DAT root length of both test varieties was less in plants grown at high level of N than the plants grown in standard culture solution and at low level of N. Difference in the root length among different treatments became more evident with increase in plant age. This might be one of the major causes of lodging of rice plants due to heavy doses of nitrogenous fertilizers.

Biomass of rice plants grown at different levels of N was recorded on the basis of fresh weight as well as dry weight of shoot and root separately (Table 2). Shoot weight of rice plants increased with increase in

Table 1. Effect of Nitrogen on the growth of rice plant

N (ppm)	Plant height (cm)		No. tillers/plant		Root length (cm)	
	TNI	IR2035	TNI	IR2035	TNI	IR2035
10 days after transplanting						
5	27.2c	23.6b	1.3c	1.2b	10.5a	15.0a
40	30.7b	23.3b	2.0b	1.8a	10.6a	15.1a
200	32.6b	24.6a	2.4a	1.7a	10.4a	14.3b
20 days after transplanting						
5	31.5c	34.1c	3.5c	3.8c	21.3a	36.0a
40	18.3b	48.9b	8.0b	8.1b	16.3b	21.2b
200	49.3a	50.1a	8.2a	8.7a	12.3c	19.3c
40 days after transplanting						
5	39.1c	47.8c	3.8c	4.1c	30.1a	50.8a
40	64.9b	73.8b	10.1b	10.1b	30.0a	47.8a
200	71.9a	79.3a	14.1a	13.3a	14.5b	20.2b

Means followed by a same letter do not differ significantly at 0.05 by LSD

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Figure 1. Effect of nitrogen on root-shoot ratio of rice plant

the application of N. Results of present investigation support the earlier studies by Yoshida (1981), Sarkar et al., 1998 and Hari et al., 1998. However, weight of roots was significantly high in the plants grown in standard culture solution than the plants grown at low and high level of N. Increase in the fresh weight of shoot was of more magnitude with increase in N from 5ppm to 40ppm than the increase of N from 40ppm to 200ppm in the culture solution. Increase in N from 40 mg/l to 200 mg/l did not give corresponding increase in dry weight in IR2035-117-3 and no significant increase in TNI (Figures 1 and 2).

Root-shoot ratio was significantly low in the plants grown at low level of N than the plants grown in standard culture solution of both test rice cultivars (Table 2). Increase in N from 40 mg/l to 200 mg/l increased root - shoot ratio in TNI and IR2035-117-3 but increase was not significant in TNI on the basis of dry weight.

Chemical Composition

Nitrogen content in the leaf sheath of rice plants increased with increase in the application of N (Table 3). However, differ-



Figure 2. Effect of nitrogen on shoots of rice plant

Table 2. Effect of Nitrogen on the biomass of rice plant

N (ppm)	Shoot		Root		Root: Shoot	
	TNI	IR2035-117/3	TNI	IR2035-117/3	TNI	IR2035-117/3
Fresh weight/plant (g)						
5	11.2c	9.7c	13.1c	12.8c	0.87c	0.77c
40	84.7b	71.1b	40.7a	43.8a	2.08b	1.62b
200	102.8a	96.3a	31.0b	36.7b	3.32a	2.63a
Dry weight/plant (g)						
5	3.0b	2.5c	1.7c	1.6c	1.80b	1.60c
40	18.8a	14.4b	5.3a	5.7a	3.56a	2.51b
200	18.1a	16.9a	4.4b	4.8b	4.09a	3.54a

Means followed by a same letter do not significantly differ at 0.05 by LSD

ent levels of N, in culture solution did not alter the contents of P, K, Mg, Ca and total free sugars in the leaf sheaths of rice plants. Our results differed from those of Sarkar et al. (1998) who stated that application of N increased P. The difference in the results might be attributed to differences in the experimental conditions and those of rice varieties. Carbon and soluble proteins increased with increase in the level of N in the culture solution. However, the content of Si decreased drastically with increase in the application of N. Rice plants grown at low level of N had comparatively high content of Zn than the plants grown at high level of N. The results of present investigations agree with Yoshida and Tanaka (1969), who stated that symptoms due to Zn deficiency were aggravated by

increased applications of N as the rice plants required more Zn at high N levels.

Although C content in the rice plants increased with increase in N application but increase in N content was more. The C:N decreased considerably with increase in the quantity of N in the culture solution. Hoff et al. (1974) stated that N fertilization had the greatest effect on the levels of soluble N compounds. The most remarkable change related to the increase in soluble N content is the decline in C:N. Rice plants grown in the high-N solution had N 1.4 times as much as the plants in the low N solution and C:N were 13 in high-N plants but 20 in the low-N ones (Ishii and Hirano, 1959). In the present investigations C:N in TNI were 65, 38 and 13 respectively at 5, 40 and 200ppm. The re-

Table 3. Effect of Nitrogen on the chemical composition of rice plant

Constituents	TNI				IR2035-117-3			
	5	40	200	400	5	40	200	400
N (%)	0.57	1.03	3.11	4.42	0.64	1.03	3.09	3.72
P (%)	0.29	0.29	0.23	0.33	0.39	0.31	0.30	0.40
K (%)	1.69	1.87	1.17	1.70	1.97	2.05	1.44	2.00
Mg (%)	0.14	0.20	0.15	0.15	0.21	0.23	0.20	0.20
Ca (%)	0.09	0.07	0.06	0.07	0.09	0.08	0.06	0.09
Si (%)	3.72	2.18	1.98	0.31	4.57	2.57	2.36	0.34
C (%)	37	39	40	41	36	38	39	42
Zn (ppm)	23	14	8	11	26	12	11	11
Fe (ppm)	25	44	68	458	32	52	101	208
C : N	65	38	13	9.3	56	37	13	11.3
SP (%)	3.57	3.40	5.78	5.74	3.42	3.37	5.32	5.78
TFS (%)	3.66	3.69	2.99	3.52	2.08	3.38	2.15	2.86

SP = soluble protein; TFS = total free sugars

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spective figures for IR2035-117-3 rice cultivar were 56, 37 and 13.

Host-Insect Interactions

The magnitude of insect population on plants is determined by complex, dynamic and variable plant-insect interactions (Whittaker, 1970). In our studies significantly more *S. furcifera* females were attracted to and settled on plants with increase in the quantity of N in the culture solution for raising rice plants (Table 4). It means increase in the application of N made the plants more attractive to the insect.

Intake and assimilation of *S. furcifera* adult females increased significantly with increase in the level of N in the culture solution for growing rice plants (Table 5).

Our results agree with Scriber (1984) who stated digestion capacity increased at higher levels of N in the food.

Growth and development of *S. furcifera* increased with increase in the application of N to rice plant (Table 6). Nymphal survival of the insect was significantly lower on N-deficient rice plants than at high level of N on both resistant and susceptible rice varieties. However, Pablo (1977) reported that survival of *S. furcifera* nymphs increased at higher level of N only on resistant cultivars. Nymphal duration of the insect was significantly higher at low level of N (5 ppm) than on plants raised on higher level of N (200 ppm) on test rice plants.

Adult life of *S. furcifera* males and females increased with increase in the amount of N application to rice plants (Table

Table 4. Effect of different levels of N in the culture solution on the orientational and settling response of *S. furcifera*

Treatment	Orientation	Setting
TNI (5 ppm) vs TNI (40 ppm)	67.8**	164.3**
TNI (200 ppm) vs TNI (40 ppm)	57.7**	56.2**
IR 2035 (5 ppm) vs IR 2035 (40 ppm)	33.2**	287.2**
IR 2035 (200 ppm) vs IR2035 (40 ppm)	161.2**	53.9**
Chi square tests were performed to determine whether observed ratios of females attracted to less N and excessive N and control plants (40 ppm N) deviated significantly from the no preference ratio of 1:1		
**Significant at 0.01 level by chi square		

Table 5. Effect of different levels of N in culture solution on intake and assimilation of food by *S. furcifera*

N (ppm)	TNI	IR2035-117-3	DIFF.
Food intake (mg)/female/24 h			
5	6.0c	3.1b	2.9**
40	10.0b	4.8a	5.2**
200	11.7a	5.7a	6.0**
Food assimilated (mg)/female/24 h			
5	0.45b	0.14b	0.31**
40	0.53ab	0.21ab	0.32**
200	0.55a	0.23a	0.32**
Honey dew excreted (mg)/female/24 h			
5	5.6c	2.9b	2.7**
40	9.5b	4.6a	4.9**
200	11.1a	5.5a	5.6**

Means followed by the same letter do not differ significantly at 5% level by DMRT

**Significant at 1% level by LSD

Table 6. Effect of different levels of Nitrogen in culture solution on the growth and development of *S. furcifera*

N (ppm)	TNI	IR2035-117-3	Diff.
Nymphs becoming adults (%)			
5	75.0b	15.0b	60.0**
40	95.0a	22.5ab	72.5**
200	97.5a	32.5a	65.0**
Nymphal duration (days)			
5	15.6a	19.5a	-3.9**
40	15.1ab	19.0ab	-3.9**
200	14.7b	18.7b	-4.0**
Growth index			
5	4.83b	0.79b	4.04**
40	6.30a	1.19b	5.11**
200	6.66a	1.74a	4.92**

Means followed by the same letter do not differ significantly at 5% level by DMRT

** = significant at 1% level by LSD

7). Likewise fecundity of females was significantly higher on rice plants raised at higher level of N than those rice plants raised at low level of N (Table 7). However, the difference in the fecundity at 5 ppm vs. 40 ppm N or 40 ppm vs. 200 ppm N in case of susceptible rice plants was non-significant. Results of our study were in conformity with other scientists (Kalode, 1974; Pathak, 1975; Dyck et al., 1979) in case of

brown planthopper, *Nilaparvata lugens*. In their studies the fecundity of the insect increased with increase in the amount of N to rice plants. Adult longevity and fecundity of *S. furcifera* showed significant interaction between variety and N levels.

Increase in the quantity of N in culture solution increased population of insect more (Table 8). However, the magni-

Table 7. Effect of different levels of Nitrogen in culture solution on adult longevity and fecundity of *S. furcifera*

N (ppm)	TNI	IR2035-117-3	Diff.
Male longevity (days)			
5	12.2c	4.3c	7.9**
40	13.9b	5.0b	8.9*
200	14.9a	8.1a	6.8**
Female longevity (days)			
5	18.4c	6.6c	11.8**
40	20.5b	7.9b	12.6**
200	22.1a	13.2a	8.9**
Fecundity (no. of eggs/10 females)			
5	903b	19c	884**
40	1079ab	88b	991**
200	1170a	194a	976**

For fecundity the analysis is based on values transformed to $\log(x + 1)$. Means followed by the same letter do not differ significantly at 5% level by DMRT

** = significant at 1% level by LSD

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Table 8. Effect of different levels of N in the culture solution on the population build up of *S. furcifera*

N (ppm)	TNI	IR2035-117-3	Diff.
5	312c	6c	306**
40	1619b	21b	1598**
200	2184a	58a	2126**

tude of population increase was greater on resistant rice plants than on susceptible ones. According to Chauvin (1967) increase in N content was linked with increase in protein which are important in herbivore nutrition. Suitability of host plant was positively correlated to its N content (Mattson, 1980).

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