Effect of Nitrogen on Water Content, Sap Flow, and Tolerance of Rice Plants to Brown Planthopper, *Nilaparvata lugens*

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**Abstract:** Water content (WC) and sap flow from leaf sheath of rice plants with varying nitrogen levels at different growth stages, and fluctuations in relative water content (RWC) of rice plants being damaged by brown planthopper (BPH), *Nilaparvata lugens* were determined in the laboratory, and the tolerance of rice plants to BPH at different nitrogen regimes was evaluated in the greenhouse at International Rice Research Institute (IRRI), the Philippines. The results indicated that both WC and RWC were increased significantly, as the amount of sap flow from rice plants was reduced statistically, with the increase of nitrogen content in rice plants. RWC in rice plants applied with high nitrogen fertilizer decreased drastically by the injury of BPH nymphs, while the reduced survival duration of rice plants with the increase of nitrogen content was recorded. These may be considered to be one of the important factors in increasing the susceptibility to BPH damage on rice plants applied with nitrogen fertilizer.

**Key words:** brown planthopper; nitrogen; relative water content; sap flow; tolerance

The Green Revolution was characterized by the increased inputs of water, pesticides, fertilizers and high yields [1], but has also resulted in some hazards such as the brown planthopper (BPH), *Nilaparvata lugens* Stål, shifting from a minor to major insect pest in Asia [2, 3]. These agronomic practices mainly included applications of high doses of nitrogen fertilizer and broad-spectrum insecticides [4, 5]. Research showed that rice plants, that had a high application of nitrogen, tended to boost up BPH activities as survival rates [6], increased fecundity [7], feeding rates and higher honeydew excretion [8]. The combined effects of increase colonization and improved performance may result in rapid population growth and high densities in nitrogen enriched rice [9,10]. Thus nitrogen fertilization had been implicated as a cause of BPH outbreaks and a threat to the rice industry in the 1970s and 1980s [3,9].

Nitrogen content is regarded as an indicator of plant quality and is also one of the most important performance limited factors of herbivores [11]. The advancing BPH population may be due to an increase in soluble amino acid content in rice sap that increases nutritional value [6, 10,12]. Other factors such as water content and nutrient sources are also important ones [13]. However, studies on the relationships between rice plants’ water content and BPH tolerance particularly with different nutritional status are limited. In this paper, we report the influences of nitrogen input on plant water content and sap flow, and their effects on rice tolerance to BPH.

**MATERIALS AND METHODS**

**Rice plants growth and brown planthopper cultures**

All BPH cultures were maintained on standardized host plant materials. Three to four 10-day-old rice seedlings of Taichung Native 1 (TN1, BPH susceptible) and IR64 (moderate resistance to BPH) were transplanted into clay pots filled with garden soil fertilized with nitrogen fertilizer, ammonium nitrate, applied at 7 days after transplanting (30%), tillering (30%) and reproductive stage (40%), respectively. Plants with four nitrogen regimes of 200, 100, 50 and 0 kg/ha were used to have a change in nitrogen content. The amounts of fertilizer input applied to each pot were calculated based on the amount of soil in each pot. All cultures and experiments were maintained in a green house with room temperature ranging from 25–40℃, RH
of 70–90% and light periods of 12 hours(light) : 12 hours(dark).

BPH adults collected from rice fields in Laguna, the Philippines, placed in an oviposition cage with TN1 plants, were used to start insect cultures. On every Monday and Thursday, 45–60 days old plants in pots were placed in the cages for 24-hour egg oviposition.

**Calibration of chlorophyll meter readings with tissue nitrogen**

An electronic chlorophyll meter (SPAD-502, Minolta camera Co., Osaka, Japan) was calibrated to assess tissue nitrogen content. At different growth stages, eight uppermost fully expanded leaves were selected randomly from plants in each nitrogen regime for SPAD meter readings. The tillers bearing these leaves were then removed, placed separately in brown paper bags in an oven at 110°C for 30 min followed by 80°C for 48 h. Nitrogen content was assessed by micro-Kjeldahl digestion and distillation. A linear model of tissue nitrogen content (%) and SPAD meter reading was established to converse SPAD meter readings to nitrogen contents.

\[ N = 0.1151 \times SPAD - 1.2772 \]  
\[ R^2 = 0.6532, F=162, P<0.001 \]

Where N is the percent of nitrogen content and SPAD is the SPAD meter reading.

**Measurement of water content in rice plants**

The relative water content (RWC) was measured by cutting 5–10 cm² mid-leaf, and then subsequently placing in a pre-weighed airtight vial (Dia. 2.5 cm, height 10 cm). After weighing the vials to obtain leaf sample weight (W), the leaf samples were immediately hydrated by distilled water up to full turgidity in the refrigerator at about 10°C for 4 h. They were then taken out of water, well dried with filter paper and weighed to obtain the fully turgid weight (TW). The samples were then dried at 80°C for 24 h and weighed to calculate the dry weight (DW). All weighing operations were performed on a 0.1-mg sensitivity balance (Mettler AJ156) and \( RWC = \frac{(W-DW)}{(TW-DW)} \times 100\% \)

**Determination of the changes in RWC of infested rice plants**

To determine the effects of BPH damage on the changes in RWC of rice plants from different nitrogen regimes, 35-day-old potted rice plants, trimmed to two main tillers, were covered with mylar cages and infested with 100 or 200 3rd instar BPH nymphs. BPHs with no treatment were maintained as control. RWC was measured at 2 and 5 days after BPH infestation. Each treatment was replicated five times.

Quantification of sap flow from leaf sheath of rice plants

The second outer leaf sheath was cut in the middle with scissors sterilized with 70% alcohol. The cut leaf was covered by a dried test tube (Dia. 1.2 cm, height 6 cm) with a pre-weighed filter paper (\( W_1 \)) to absorb the sap leaching out of leaf cut, and the tube was then sealed using parafilm®. The filter paper in test tube was replaced by another pre-weighed one and weighed (\( W_2 \)) again. The amount of sap flow was calculated as the difference between \( W_1 \) and \( W_2 \).

**Rice plants bioassay**

Two hundred 2nd instar nymphs maintained on TN1 were introduced into cages containing 35-day-old potted rice plants trimmed to 6 tillers. SPAD meter readings were recorded before experiment and at 15th day after infestation by BPH, respectively. Damages to the rice plants were rated daily based on the Standard Evaluation System for Rice. In the experiment on plant tolerance to damage by BPH nymphs, the number of nymphs left in each cage was checked at 3-day interval. The same status nymphs reared on TN1 plants were supplied into cage to keep the relative constant density of about 200 nymphs in each cage.

**Statistics analysis**

Linear relationships were computed using IRRISTAT Ver. 4.0 (IRRI, 2000). Analysis of variance (ANOVA) and Duncan’s Multiple Range Test were performed with the SAS package using PROC ANOVA or PROC GLM.

**RESULTS**

**Water content in rice plants with different nitrogen content**

Both water content (WC) and relative water content (RWC) in rice plants at different growth stages were significantly (\( P<0.001 \)) correlated to nitrogen content, and the increasing trends regressed at three rice growth stages were almost parallel lines (Fig. 1 and Fig. 2). However, the slopes of the regression lines in RWC were higher than in WC at all three stages. This might be due to greater sensitivity to the increased nitrogen content in RWC as compared to WC.

**Changes in RWC of rice plants damaged by BPH**

RWCs in 35-day-old rice plants at different nitrogen regimes were measured at 2 and 5 days after infestation by 3rd instar nymphs under the densities of 100 and 200 BPH per plant. Results highlighted that
Fig. 1. Water content of rice plants with different nitrogen content.
▲ 35-day rice plants ($R^2=0.4339$, $df=23$, $F=16.86$, $P=0.0005$)
● 45-day rice plants ($R^2=0.8198$, $df=23$, $F=100.1$, $P<0.0001$)
■ 55-day rice plants ($R^2=0.5586$, $df=23$, $F=27.84$, $P<0.0001$)

Fig. 2. Relative water content of rice plants with different nitrogen content.
▲ 35-day rice plants ($R^2=0.6350$, $df=23$, $F=38.27$, $P<0.0001$)
● 45-day rice plants ($R^2=0.5264$, $df=24$, $F=24.45$, $P<0.0001$)
■ 55-day rice plants ($R^2=0.6637$, $df=23$, $F=43.42$, $P<0.0001$)

Fig. 3. Relative water content of rice plants damaged by BPH.

RWC in BPH-free plants with high nitrogen fertilizer (200 kg N/ha, 200N) was significantly higher than that with no nitrogen fertilizer (0 kg N/ha, 0N) ($P=0.0155$, Fig. 3), and the average RWC both in 200N and 0N plants maintained a relative constant value during the 5-day experiment ($P=0.9128$). RWC in 200N rice plants drastically decreased with the injury by 100 and 200 BPH nymphs ($P=0.0387$) and with the prolonged damage by BPH ($P=0.0257$) compared to those in BPH-free plants. On the contrary, RWC in 0N rice plants rose slightly with the injury by 100 and 200 BPH nymphs compared to control. However, no significant differences were detected in different treatments in both 2 days ($P=0.0836$) and 5 days ($P=0.2413$) after infestation.

Fig. 4. Sap flow from leaf sheath of rice plants with different N content within 72 hours.
▲ 35-day rice plants ($R^2=0.0273$, $F=0.393$, $P=0.5473$)
● 45-day rice plants ($R^2=0.3755$, $F=13.23$, $P=0.0015$)
■ 55-day rice plants ($R^2=0.4361$, $F=14.69$, $P=0.0012$)

Sap flow from leaf sheath of rice plants

The total amount of sap collected from the leaf sheaths at different growth stages within 72 h was regressed with the nitrogen content of rice plants (Fig. 4). It pointed out that the sap flow from 35-day-old rice plants was not, in fact, obviously related to the nitrogen...
Fig. 5. Rate of sheath sap flow from 35-day-old rice plants with different N fertilization.

Fig. 6. Rate of sheath sap flow from 55-day-old rice plants with different N fertilization.

Fig. 7. Duration from infestation to 9th grade damaged by BPH of rice plants.

Fig. 8. Dynamics of grade damaged by 200 2nd instar nympha of rice plants.

content of rice plants ($P>0.05$), while those from 45- and 55-day-old plants were decreased significantly with the increase of nitrogen content in rice plants ($P<0.001$). It resulted in a shift in the order of sap amount from 55 > 45 > 35 in low nitrogen to 55 < 45 < 35 in high nitrogen plants, and the intercrossed point of the three regressed lines was located at 2.4% nitrogen content (Fig. 4), where the rate of nitrogen fertilizer applied to rice plants was 50 – 100 kg/ha.

The amount of sap from leaf sheaths within 72 h may be attributed to the rate of sap flow at different duration intervals. In 35-day-old rice plant (Fig. 5), the rate of sap flow within 24 h was significantly higher in 200N rice plants than in 0N rice plants, more than two times higher. However, the rate was dropped sharply by 10 times in 200N rice plants after 25 h, while it increased slightly in 0N rice plants. The average rate of sap flow in 200N rice plants was not obviously different with that in 0N rice plants during 72 h ($P>0.05$). Compared with the 35-day-old rice plants, the rate of sap flow in 200N 55-day-old rice plants exhibited a higher trend than in 0N rice plants only within 6 h, and it declined after 25 h (Fig. 6), while it increased significantly in 0N rice plants during 72 h, with the effect that the average rate of sap flow was significantly higher in 0N rice plants than in 200N rice plants ($P<0.0001$).

Tolerance to BPH injury of rice plants with different nitrogen regimes

Tolerance of rice plants to BPH injury varied considerably among rice plants with respect to different nitrogen content. The survival durations of rice plants, or the duration from infestation with BPH to the death of rice plants, declined significantly with the increase of nitrogen content in rice plants damaged by both 15 pairs
of adults and 200 2nd instar nymphs (Fig. 7). However, much shorter survival durations were recorded in these plants infested by nymphs than those by adults.

The damage assessments of rice plants infested by 200 2nd instar nymphs of BPH fluctuated more greatly in the plants applied with 200 kg/ha nitrogen fertilizer than those with 100 kg/ha and 0 kg/ha (Fig. 8), implying that there were higher individual differences among rice plants with high nitrogen fertilizer. While sharp increments of damage were obtained in plants with high nitrogen, gradual increases were recorded in the unfertilized plants. Meanwhile, smaller body sizes and higher mortality of nymphs and adults were observed on the unfertilized rice plants compared to those with 100 and 200 kg/ha nitrogen fertilizer (data unpublished).

**DISCUSSION**

RWC is the appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit. While water potential as an estimate of plant water status is useful in dealing with water transport in the soil-plant-atmospheric continuum, it does not account for osmotic adjustment (OA). OA is a powerful mechanism of conserving cellular hydration under drought stress and RWC expresses the effect of OA in this respect. Hence, RWC is an appropriate estimate of plant water status in terms of cellular hydration under the possible effect of both leaf water potential and OA [15]. In rice, the typical RWC at wilting is in the range of 60% to 70%. Rice plants with high nitrogen fertilizer showed a reducing trend significantly in their RWC at 5 days after infestation by high density of BPH nymphs compared to unfertilized plants (Fig. 3). The RWC of 68.89% in rice plants damaged by 200 nymphs for 5 days had been in the wilting scale of RWC. It implied that the characteristics of BPH injury to rice plants were related closely to the RWC of plants. The mechanisms of “hopperburn” of rice plants may be similar with those of drought stress. Since drought stress resulted in the lower osmotic potential and higher concentration of such nitrogenous compounds as amino acids, nitrates and betaine in plant tissues [16], and the higher abundance of herbivores also [17].

The change in RWC of rice plants was in connection to the rate of sap flow, because rate of sap flow reduced sharply after 24 h in rice plants with higher nitrogenous fertilizer than those with non-nitrogenous fertilizer (Fig. 5). It may be explained as that rice plants with higher nitrogen content bearing higher water potential can provide hoppers at low density with enough sap for their higher survival and faster growth. However, at higher density of BPH, the RWC of plants reduced sharply, and the plants wilted were attributed to the amount of sap sucked by high dense hoppers that exceeded those supplied by rice plants due to the slower rate of sap flow from host plants. Water-stressed plants show increased levels of amino acids, particularly an increase in proline and soluble carbohydrates [18]. Hence, water-stressed plants conditioned with enhanced nutrients, together with less effective qualitative defensive chemicals, can be more attractive to herbivores and suffer from increased levels of feeding [19].

The resistance of rice plants to BPH decreased by an enhanced application of nitrogenous fertilizer, has been reported in variety studies [8, 20]. The increasing susceptibility of rice plants to BPH was related remarkably to higher feeding activities of BPH on plants with high nitrogenous fertilizer [6, 7]. Lower sap flow in rice plants may be another important mechanism of susceptibility to BPH injury under a high dosage of nitrogen fertilizer.

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**REFERENCES**


