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Effects of Nitrogen Applications on Transpiration, Physiological, and Growth Characteristics of Sugarcane (*Saccharum spp.*)

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Abstract

Improving fertilizer and water use efficiencies is an important strategy in agricultural production. This study aimed to evaluate the effect of nitrogen fertilization on the daily whole-plant transpiration, growth, and physiological activities of sugarcane. The pot experiment was conducted under glass-house conditions. Five treatments of various nitrogen application rates (0, 4, 8, 12, and 16mM) were assigned in a randomized complete block design with three replications. The result showed that higher nitrogen applications led to higher growth, physiological, and biomass parameters. Nevertheless, biomass nitrogen use efficiency and photosynthetic water use efficiency declined because of applying nitrogen at higher rates. During the first weeks of plant growth, no significant differences in plant height, leaf number, SPAD, and whole-plant transpiration among nitrogen treatments indicated that higher nitrogen added was not necessary during this time. Nitrogen and water use efficiencies could be improved if the remaining soil nitrogen and crop growth stage nitrogen requirements are estimated in cultivation practice.

Keywords

Nitrogen, photosynthesis, sugarcane, transpiration, water

Introduction

Sugarcane (*Saccharum spp.*) is an important cash crop that contributes 80% of global consumption and 40-50% of the world's bio-ethanol production (Zuurbier & Vooren, 2008). Moreover, sugarcane produces a huge biomass production annually, therefore, it requires a lot of nutrients (especially nitrogen) and water for growth, biomass accumulation, and sugar synthesis.

About 97 to 99% of water absorbed by roots is used for transpiration. Transpiration enables the mass flow of mineral nutrients to stimulate nutrient uptake, allows CO_2 diffusion to support

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photosynthesis, and brings down plant temperatures (CID Bio-Science, 2021). Being a C_4 plant, sugarcane has a photosynthetic ability superior to C_3 plants (Kajala *et al.*, 2011). Endres *et al.* (2010) reported a close relationship between photosynthesis and transpiration in sugarcane. This means that sugarcane also needs more water for transpiration. Nitrogen (N) is involved in many critical processes in plant growth, expansion of green leaves, and tiller production, especially in the formation of plant protein which is essential for photosynthesis. Previous studies showed that higher leaf N content resulting from higher N application supports better photosynthesis in sugarcane (Sage *et al.*, 2014). The N requirement of sugarcane is the greatest during tillering and the early grand growth phases (Bachchhav, 2005). Application of more N promotes late tiller formation, reduces sugar recovery, increases N in juice, and attracts pests and diseases (Castro *et al.*, 2017). Therefore, in sugarcane production, N is often supplied during the early growth stage from planting to elongating.

Water shortage frequently occurs during the early growth stage of sugarcane because of crop season management (Dinh *et al.*, 2017). Fertilizer application with high doses of N during this stage supports stronger photosynthesis and also induces more transpiration. This agricultural practice implicits more risks from water stress if the water supply is limited. Hence, applying N efficiently to support sugarcane growth and using water sources sparingly are important strategies in sugarcane cultivation. Previous studies mostly investigated the effects of N on photosynthesis and the growth of sugarcane, whereas little information on transpiration, especially daily whole plant transpiration, has been found. Therefore, this study focused on daily transpiration, growth, and physiological responses to different N applications. The results of the study would provide useful information to enhance N use efficiency in sugarcane at the early growth stage.

Materials and Methods

The experiment was conducted under glasshouse conditions at the University of the Ryukyus, Okinawa, Japan (26°25’ N, 127°45’ E; altitude 126m) with the daily average temperature, relative humidity, and solar radiation ranging from 13.3 to 26.2^{o}C, 35.7 to 88.5%, and from 30.1 to 317.4 W m^{-2}, respectively. The free-disease eight-month-old plants of commercial cultivar NiF8 were cut into single bud setts and grown in containers for two months. Then, the two-month-old seedlings were transplanted into Wagner pots (Φ256 x Φ234 x 297mm) filled with 8kg substrate of red soil, sea sand, and peat moss (1:1:1, v v^{-1}). As soon as the seedlings were transplanted, irrigation with full available water was practiced. Then, water was supplied by daily water loss which was calculated by the balance method with an electronic digital scale until the end of the experiment. The modified Hoagland’s nutrient solution (500 mL) containing 6mM $CaCl_2$, 2mM K_2SO_4, 2mM KH_2PO_4, 2mM $MgSO_4.7H_2O$, 25µM H_3O_3, 10µM $MnSO_4.5H_2O$, 2µM $ZnSO_4.7H_2O$, 0.5µM $CuSO_4.5H_2O$, and 0.1mM $C_{10}H_{12}FeN_2NaO_8.3H_2O$ was fertilized weekly by replacing water irrigation. N in the form of ammonium nitrate (NH_4NO_3) was added into the nutrient solution at five different N levels of 0, 4, 8, 12, and 16mM. The experimental design was a randomized complete block design with three replications. Experimental pots were arranged between the plants and rows at 40cm and 90cm, respectively.

Data collection

The experimental pots were weighed daily by an electronic digital scale at 6pm. The pot weight before and after irrigation was recorded. The total water loss was calculated as follows:

$$WS = P1 – P2$$

Where WS is the daily water loss (g day^{-1}), P1 is the total pot weight after irrigation on the day before; and P2 is the total pot weight before irrigation on the day after.

A bare soil pot was prepared to determine daily soil evaporation as follows:

$$SE = S1 – S2$$

Where SE is the soil evaporation (g day^{-1}) from bare pot; S1 is the pot weight after irrigation on the day before; and S2 is the pot weight before irrigation on the day after.

The plant transpiration (g day^{-1}) was calculated as follows:

$$TE = WS - SE$$

Where TE is the daily plant transpiration.

From 3 weeks after transplanting (WAT), growth parameters were weekly determined. Plant height was measured from ground to top visible dewlap leaf. Leaves were marked to count the number of green leaves and the total number of leaves from the beginning to the measured times. The soil plant analysis development (SPAD) index was measured at the first fully expanded leaf by a SPAD 502 Plus Chlorophyll Meter (Konica Minolta, Japan).

At the end of the experiment (12 WAT), photosynthesis parameters, including potential photosynthesis rate, stomatal conductance, and transpiration rate, were measured at the first fully expanded leaf using LI-6400 portable photosynthesis system (LI-COR, Lincoln, Nebraska, USA) equipped with a $2x3cm^2$ LED chamber between 1100 to 1300 at a photon flux density of 2,000 μmol m^{-2} s^{-1}, leaf temperature of 33 ± 2°C, the CO_2 concentration of 400 ± 5 μmol mol^{-1}. Following that, the first leaf was cut to determine the leaf area using LI-3100 portable leaf area meter (LI-COR, Lincoln, Nebraska, USA). The extraction with 99% ethanol of a 4-cm^2 leaf sample measured the absorbance as 649 and 665nm by UV-VIS Spectroscopy to calculate the chlorophyll concentration. The remaining leaves were cut to determine the leaf area. Separated parts of the sample plants, including the first leaf, remaining leaves, stem (after squeezing), and root (after cleaning with tap water), were oven-dried at 80°C for 48h to determine dry weight. After grinding by TI-100 vibrating sample mill (CMT, Tokyo, Japan), 25mg of dry samples was taken to determine nitrogen content using an N/C analyzer (NC-90A, Shimadzu, Japan). Juice samples were used to analyze the Brix and electrical conductivity by Atago PAL-1 Digital Pocket Refractometer and EC-33 Horiba Laquatwin Conductivity Meter, respectively. The juice samples after being stored at -80°C were completely melted, and concentrations of major ions in sugar juice were determined by Ion Chromatography (ICS-1600, Thermo Fisher Scientific).

Photosynthetic nitrogen use efficiency (PNUE) and photosynthetic water use efficiency (PWUE) were then calculated using the following formula:

PNUE = Photosynthetic rate/total leaf nitrogen content;

PWUE = photosynthetic rate/transpiration rate.

Biomass nitrogen use efficiency (BNUE) and water use efficiency (BWUE) were then calculated by the following formula:

BNUE = total biomass production/ nitrogen applied amount

BWUE = total biomass production/ total water application amount

Statistical analysis

The data were subjected to analysis of variance according to a Randomized complete block design using Statistix version 8.0. Tukey test was used to compare the means.

Results

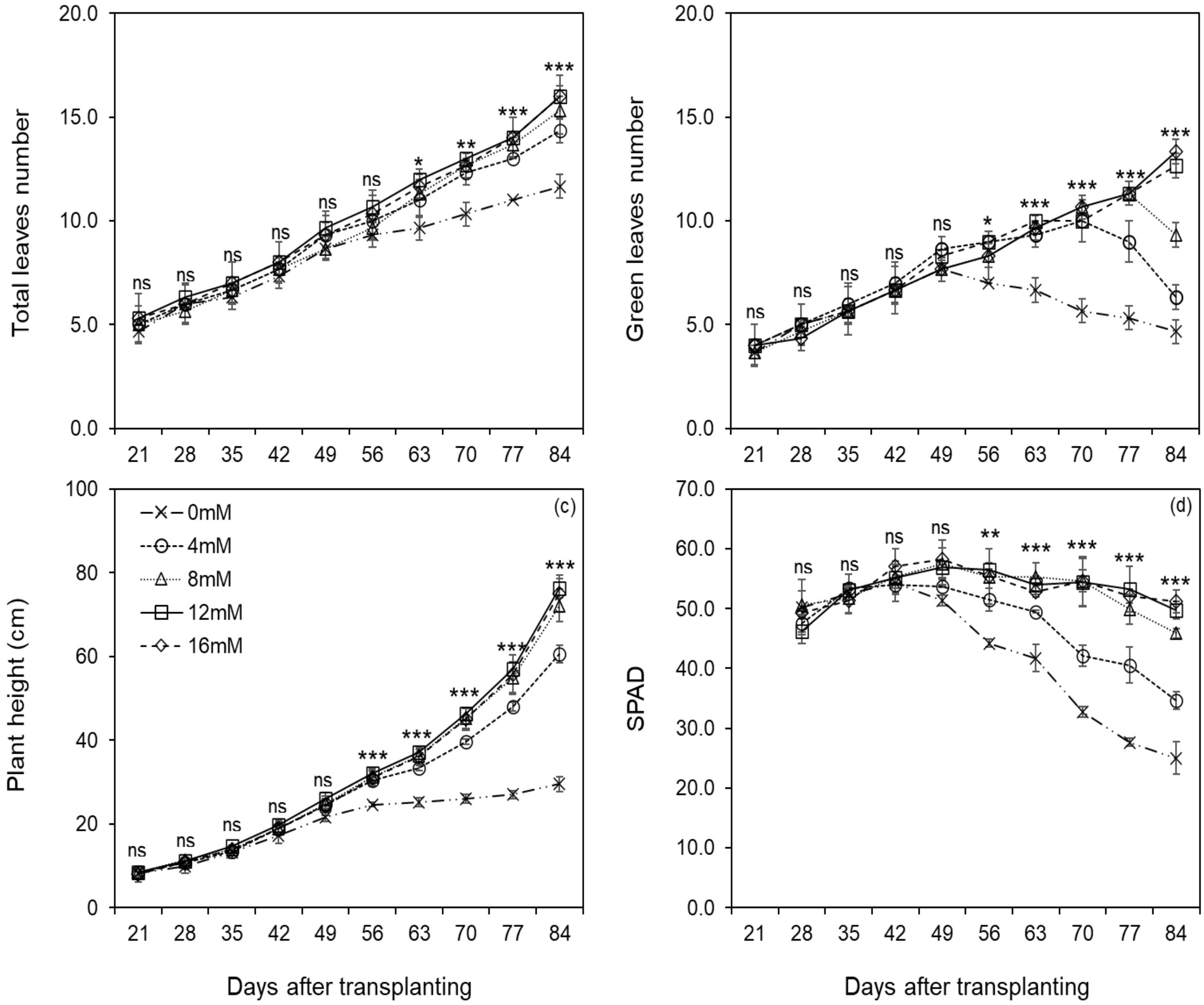
Growth of sugarcane under different nitrogen applications

There were no significant differences among N application levels in the total number of leaves until 63 days after transplanting (DAT). At this time, the increasing rate of the total number of leaves of the 0mM treatment was slower than in other treatments. However, a difference was found between 0mM and the highest N application treatments (12mM and 16mM). From 70 DAT, the total number of leaves of the 0mM treatment was significantly lower than in the other treatments. The difference between 4mM and higher N application treatments was found until 84 DAT (**Figure 1a**). The number of green leaves increased during the experimental period except for the reduction in the 0-mM, 4-mM, and 8-mM treatments from 49, 70, and 77 DAT, respectively. The difference was found earlier in the number of green leaves at 56 DAT with the lower value of the 0-mM treatment compared to other treatments. The significantly lower values in the number of green leaves of the 4-mM and 8-mM treatments than higher N application treatments started from 77 and 84 DAT, respectively (**Figure 1b**).

The plant height of the 0mM treatment was lower than other treatments from 42 DAT but became significantly lower only from 56 DAT. A lower plant height of the 4-mM treatment than higher N application treatments was recorded from 63 DAT (**Figure 1c**). SPAD of treatments slightly increased to the peaks of 51.4, 53.7, 57.5, 56.9, and 58.3 for the 0, 4, 8, 12, and 16-mM treatments, respectively, at 49 DAT and then decreased. SPAD of the 0-mM treatment decreased earlier with higher reduction rates from 42 DAT. The difference among N application treatments was recorded from 56 DAT with lower SPAD values of the 4-mM treatment compared to other treatments. At 84 DAT, the 8-mM treatment had significantly lower SPAD than higher N application treatments (**Figure 1d**).

Water loss and transpiration of sugarcane under different nitrogen applications

In the first 50 days, the whole plant's daily water loss and transpiration ranged from 40 to 260 g day^{-1} and from 5 to 90 g day^{-1}, respectively, without significant differences among treatments. During this period, the highest



*Note: ns- non-significant; *, **, *** are significantly different at P <0.05, 0.01, and 0.001, respectively.*

Figure 1. The number of leaves, plant height, and SPAD value of sugarcane under different nitrogen application levels

amount of water loss came from transpiration. After that, at non-N application treatment (0 mM), daily water loss ranged from 40 to 370 g day^{-1}, while daily transpiration fluctuated from 0 to 115 g day^{-1}, significantly lower than N application treatments. The daily water loss and transpiration tended to increase during plant growth in all N application treatments. From 58 DAT, the daily water loss and transpiration of the 4-mM treatment were lower than those of the other N treatments. The 8-mM treatment had a lower water loss and lower transpiration values than the higher N-level treatments, but the difference was not significant until 76 DAT. From 81 DAT, the difference between the 12-mM and 16-mM treatments became clearer with higher values in the 16-mM treatment (**Figure 2**).

Nitrogen content in plant tissues under different nitrogen applications

N content in the 1st leaf, whole leaves, stalk, and root increased as the N application level increased. N content in leaves, especially in 1st leaf was higher than those in stalk and roots. There were no differences in N content in 1st leaf, whole leaves, and roots between the treatments of 8mM and 12mM N. The differences in N contents in whole leaves and roots between the 12mM and 16mM treatments were also not significant. Non-N application resulted in the lowest values (0.70, 0.75, 0.33, and 0.41%) in N contents of all plant parts (1st leaf, total leaves, stem, and root, respectively). However, the difference between this treatment and lower N-level treatments (4mM and 8mM) was not significant (**Table 1**).

Photosynthesis characteristic of sugarcane under different nitrogen applications

Photosynthetic rate, stomatal conductance, transpiration rate, and leaf chlorophyll content increased when the N dose increased. However, the difference was insignificant when the N dose increased from 12mM to 16mM. Internal CO_2 in the 0-mM treatment was the highest (253.9 µmol mol^{-1}), whereas no significant difference was found among N application treatments. There was also no difference in PNUE among all treatments. PWUE of N application treatments was significantly different, with the highest values found in the 4-mM and 8-mM treatments (6.4 µmol mol^{-1}), which were followed by those of the 12-mM treatment (5.5 µmol mol^{-1}) and the 16-mM treatment (5.2 µmol mol^{-1}). The no N application treatment had the lowest PWUE with only 4.7 µmol mol^{-1} (**Table 2**).

Leaf area and dry matter accumulation of sugarcane under different nitrogen applications

The first leaf area, total leaves area, and dry weights of leaf, stem, root, and whole plant increased when the N application levels increased. However, the differences in 1st leaf area, total leaves area, and leaf dry weight were not significant between the 12-mM and 16-mM

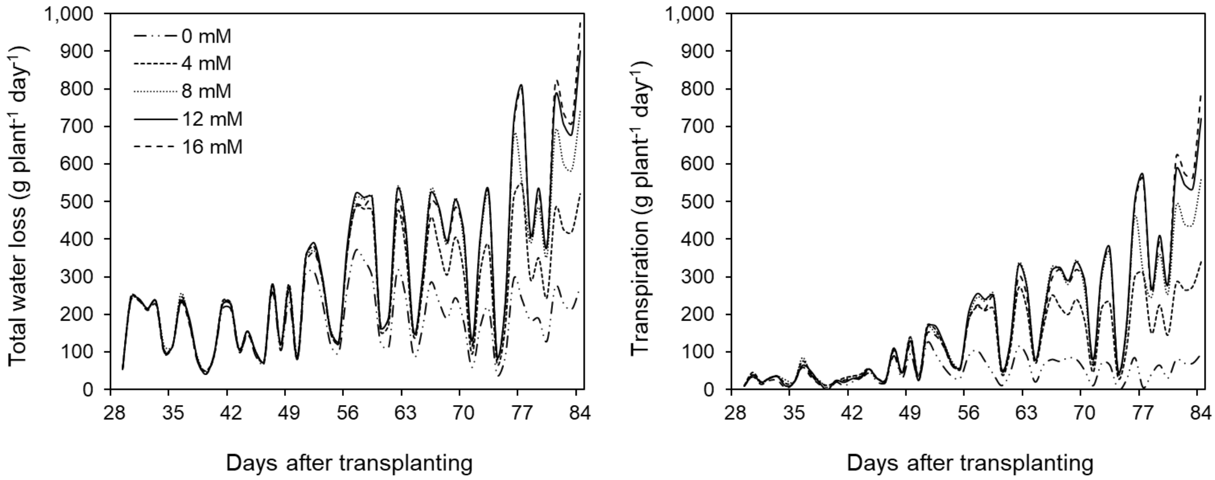


Figure 2. Whole plant water loss and transpiration of sugarcane under different nitrogen application levels

treatments, and the differences in dry weights of the stem, root, and whole plant were also not significant among the 8-mM, 12-mM, and 16-mM treatments. The increase of N applied levels from 4mM to 16mM led to a decrease in BNUE, but an increase in BWUE when. However, there were no significant differences in BWUE among the treatments of 8mM, 12mM, and 16mM N with the same values of 5.0 g g^{-1} (**Table 3**).

Ion content in sugar juice of sugarcane under different nitrogen applications

The investigation of the ion content of sugar juice showed no differences in F^- and SO_4^{2-} among all of the N-applied treatments. Meanwhile, Cl^- and PO_4^{3-} decreased and NO_3^- increased due to the increase of N application. Regarding cations, there were no differences in Na+, Ca^{2+}, and Mg^{2+} contents among the treatments. Nonetheless, increasing N doses resulted in higher content of NH_4^+ and lower content of K^+ content (**Table 4**).

Discussion

Nitrogen is involved in many critical plant processes of sugarcane such as plant growth and expansion of green leaves. When N is deficient, the plant becomes stunted and the yellowing of leaves occurs (Bell *et al.*, 2014). Dinh *et al.* (2017) found that non-N application caused stunted growth with a very slow increase in plant height and the total number of leaves from 14 DAT, whereas the difference among N-applied treatments was clear at 63 DAT. Our studies showed similar results with slower growth of 0mM and lower N-applied treatments compared to higher N-applied treatments from 42 DAT and 70 DAT, respectively. The effects of N deficiency were obviously seen from the changes in SPAD and GLNs (**Figure 1**). The symptoms of N deficiency were also shown in lower values of 1st leaf area, total leaves area, and total leaf chlorophyll content of the 0-mM and lower N application treatments (4mM and 8mM) compared to the higher N application treatments (12mM and 16mM). Other previous studies found the same results in plant height, total leaves number, leaf area, and SPAD under the effects of various N-applied levels (Wiedenfed & Enciso, 2008; Saleem *et al.*, 2012, Zeng *et al.*, 2020).

Table 1. Nitrogen contents in 1st leaf, total leaves, stem, and root of sugarcane under different nitrogen application levels

Treatment	1st leaf N (%)	Total leaves N (%)	Stem N (%)	Root N (%)
0mM	0.70^{d}	0.75^{d}	0.33^{d}	0.41^{c}
4mM	1.17^{c}	1.13^{c}	0.51^{c}	0.49^{c}
8mM	1.55^{b}	1.46^{b}	0.58^{c}	0.53^{bc}
12mM	1.69^{b}	1.68^{ab}	0.73^{b}	0.76^{ab}
16mM	1.96^{a}	1.83^{a}	0.89^{a}	0.89^{a}

Note: N- Nitrogen content. Different small letters in the same column indicate significant differences at P <0.05.

Table 2. Photosynthesis characteristics of sugarcane under different nitrogen application rates

Treatment	Photosynthetic rate (µmol m^{-2} s^{-1})	Stomatal conductance (mol m^{-2} s^{-1})	Intercellular CO_2 content (µmol mol^{-1})	Transpiration rate (mmol m^{-2} s^{-1})	Chlorophyll content (mg m^{-2})	PNUE (µmol s^{-1} g^{-1})	PWUE (µmol mol^{-1})
0mM	21.2^{d}	0.24^{c}	253.9^{a}	4.5^{c}	3.6^{d}	30.2^{a}	4.7^{c}
4mM	37.5^{c}	0.37^{bc}	201.4^{b}	5.8^{bc}	12.7^{c}	32.1^{a}	6.4^{a}
8mM	46.1^{b}	0.52^{b}	204.3^{b}	7.2^{b}	26.5^{b}	29.9^{a}	6.4^{a}
12mM	51.2^{a}	0.72^{a}	219.6^{b}	9.3^{a}	34.9^{a}	30.4^{a}	5.5^{b}
16mM	53.7^{a}	0.82^{a}	221.7^{b}	10.3^{a}	39.2^{a}	27.3^{a}	5.2^{b}

Note: Different small letters in the same column indicate significant differences at P <0.05.

Table 3. Leaf area and biomass parameters of sugarcane under different nitrogen application rates

Treatment	1st leaf area (cm)	Total leaf area (cm^2)	Leaf dry weight (g $plant^{-1}$)	Stem dry weight (g $plant^{-1}$)	Root dry weight (g $plant^{-1}$)	Total dry weight (g $plant^{-1}$)	BNUE (g g^{-1})	BWUE (g g^{-1})
0mM	164.2^{d}	634.6^{d}	8.1^{d}	7.0^{c}	9.8^{c}	24.9^{c}	-	3.1^{c}
4mM	346.2^{c}	1778.8^{c}	18.0^{c}	21.6^{b}	17.1^{b}	56.7^{b}	18.1^{a}	4.5^{b}
8mM	474.8^{b}	2740.1^{b}	26.8^{b}	32.7^{a}	20.8^{ab}	80.4^{a}	15.8^{ab}	5.0^{a}
12mM	515.3^{ab}	3220.5^{a}	32.3^{a}	35.5^{a}	20.3^{a}	88.1^{a}	11.7^{bc}	5.0^{a}
16mM	519.5^{a}	3336.3^{a}	33.1^{a}	34.2^{a}	20.5^{a}	87.7^{a}	9.1^{c}	5.0^{a}

Note: Different small letters in the same column indicate significant differences at P <0.05.

Table 4. Effects of nitrogen applications on anion and cation contents (ppm) in sugar juice of sugarcane

Treatment	F^-	Cl^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	Na^+	NH_4^+	K^+	Mg^{2+}	Ca^{2+}
0mM	0.16^{a}	17.50^{a}	0.00^{b}	4.84^{a}	5.64^{a}	0.10^{a}	0.00^{b}	20.38^{a}	1.85^{a}	1.90^{a}
4mM	0.28^{a}	12.21^{b}	0.00^{b}	1.40^{b}	5.93^{a}	0.11^{a}	0.00^{b}	15.45^{ab}	1.68^{a}	1.68^{a}
8mM	0.36^{a}	9.93^{c}	0.00^{b}	1.16^{b}	5.50^{a}	0.09^{a}	0.03^{ab}	13.06^{ab}	1.72^{a}	1.08^{a}
12mM	0.38^{a}	9.23^{c}	0.15^{a}	1.02^{b}	5.78^{a}	0.11^{a}	0.06^{a}	8.27^{b}	1.27^{a}	1.21^{a}
16mM	0.38^{a}	8.61^{c}	1.18^{a}	0.45^{c}	5.38^{a}	0.06^{a}	0.06^{a}	8.04^{b}	1.40^{a}	1.32^{a}

Note: Different small letters in the same column indicate significant differences at P <0.05.

The findings of Zeng *et al.* (2020) concur with our study that N concentration in plant tissues increased with the increasing N application rate. According to Bell *et al.* (2014), higher N concentration may be resulted from a greater uptake of N which is supported by early shoot and root vigor. In our study, at higher doses of N application, the plants grew faster which led to higher partial and total biomass compared to lower ones. Bologna-Campbell (2013) also found an increase of above-ground, below-ground parts, and the whole plant dry matter by increasing N fertilization. These supported the finding by Dinh *et al.* (2017) that a larger root system by adding N helps plants uptake more nutrients to create more biomass.

On the other hand, the opening and closing of the stomata promote upstream water which takes dissolved nutrients with them. Polley *et al.* (1999) revealed a close link between transpiration and plant N. Matimati *et al.* (2014) claimed that N regulates transpiration which has a functional role in controlling the mass flow of nutrients. In sugarcane, the positive correlation between leaf N content and stomatal conductance was reported in many previous studies (Cerqueria *et al.*, 2019; Querejeta *et al.*, 2022). Therefore, rising leaf N content by a higher N application will lead to higher stomatal conductance, transpiration, and N uptake. This can be seen with the greater NH_4^+ and NO_3^- contents in sugarcane juice (**Table 4**, Dinh *et al.*, 2018). No amounts of NO_3^- and NH_4^+ were detected in sugar juice in both non-N and low N application treatments (4mM and 8mM), which could show that there was a deficiency of N for sugarcane growth in these treatments. The results from our experiment and the study by Castro-Nava *et al.* (2020) asserted the positive effects of N application rates on photosynthetic rate, stomatal conductance, and transpiration rate. Our study also showed an increase in the daily transpiration rate during plant growth. However, from 50 DAT, the daily transpiration was unchanged in the non-N treatment and had a very slow increasing rate in the 4mM treatment. The reason may be derived from the low stomatal conductance leading to higher content of intercellular CO_2 which stagnated physiological processes when plants were subjected to N stress conditions (Dinh *et al.* 2017; 2019). Furthermore, although no study investigates whether increasing the content of K^+ and Cl^- in sugarcane juice causes a negative effect on sugarcane growth, Watanabe *et al.* (2016) reported that the dominance of K^+ and Cl^- in sugar juice was the reason for sugar reduction. Reduction in sugar accumulation could be resulted from the decline in growth and physiological activity when the concentrations of K^+ and Cl^- increased in plants because sugar was a product stimulated by the photosynthesis of sugarcane leaves.

The findings of Dinh *et al.* (2018) are in line with our study by also revealing that increasing N application did not change PNUE and BWUE while reducing PWUE and BNUE. Thorburn *et al.* (2014) showed a similar result with the reduction in BNUE by increasing N-applied doses. In this study, we focused on how to improve NUE and WUE. There was no difference between non-N application and N application treatments in plant height, leaf number, SPAD, and daily transpiration until 42 DAT, between the 4-mM and 8-mM treatments with the higher N application treatments until 49 DAT and 70 DAT, respectively. This means that the residue of N in the soil from the prior crop season is enough for the first few weeks of crop growth. N added in during these times is not necessary. The redundancy of N leads to an increase in transpiration, while the unchanged need for water in the plant could cause water waste. Furthermore, plants are also easily subject to drought stress if grown under limited water sources. Therefore, a rational strategy for fertilization will help to save both N and water to improve NUE and WUE. Dividing the amount of N into more fertilization times instead of three times as usual, is a solution, but it also takes more labor costs. Using slow-released N fertilizer (SNF) could be a better solution. Nguyen *et al.* (2012) indicated the effect of using SNF for maize with better growth, higher yield, and economic efficiency than the conventional method even with lower N application doses. Similarly, Atmodjo *et al.* (2015), Bhanuvally *et al.* (2017), and Liao *et al.* (2018) found that SNF increased NUE by preventing N loss, higher cane yield, and commercial cane sugar in sugarcane. Rathnappriya *et al.* (2022) demonstrated that controlled released fertilizer reduced N leaching from cropping systems. Hence, based on our study, to design a suitable SNF with better efficiency, it is important to determine N requirements for the crop in the growth stages as well as the remaining N in the soil before cultivation.

Conclusions

Our study indicates that the N application supported better growth and physiological parameters of sugarcane. Higher N applications (12mM and 16mM) resulted in higher plant height, leaf number, SPAD, N and chlorophyll contents, potential photosynthesis, whole plant transpiration, and biomass than those treatments with lower N applications (0mM, 4mM, and 8mM). However, the higher N applications did not change PNUE and BWUE, but reduced BNUE and PWUE. To improve NUE as well as WUE, the remaining N in the soil and N requirement in each growth stage should be paid more attention in the fertilization practice.

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