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Nitrogen use efficiency and drought tolerant ability of various sugarcane varieties under drought stress at early growth stage

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ABSTRACT

The experiment was conducted under glasshouse conditions to evaluate nitrogen use efficiency and drought tolerant ability of the five different sugarcane varieties (including NiF3, Ni9, Ni17, Ni21 and Ni22) under early growth stage from 60 to 120 days after transplanting. The results showed drought stress reduced the photosynthetic rate, growth parameters including plant height, leaf area; partial and total dry weights; and nitrogen use efficiency (NUE) traits including photosynthetic NUE, nitrogen utilization efficiency and biomass NUE of measured sugarcane varieties. The significant differences were found among varieties in growth parameters, dry weights, NUE traits and drought tolerant index (DTI). The significant positive correlations among NUE traits and DTI suggested higher NUEs could support better a tolerant ability to drought stress at the early growth stage. Because of larger contributions, DTIs for aboveground and stalk dry weight could be used as the important DTIs to evaluate drought tolerant ability in sugarcane varieties.

Abbreviations: A_{max} : potential photosynthetic rate; DAT: days after transplanting; DTI: drought tolerant index; NL: specific leaf nitrogen content; NUE: nitrogen use efficiency; NUE_b : biomass nitrogen use efficiency; NUE_u : nitrogen utilization efficiency; PNUE: photosynthetic nitrogen use efficiency; TN: total nitrogen content; TNU: total nitrogen uptake; WW: well-watered; DS: water stress.

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Drought tolerant index; nitrogen use efficiency; sugarcane; water stress

Sugarcane is the unique sugar source of countries in tropical and subtropical climates where contribute over 80% to the global sugar production. It is also considered as an important alternative and forage crop because of high dry matter yield with high digestibility (Ehara, Tsuchiya, & Takamura, 1994; Zubbier & Vooren, 2008). However, to create the optimum production, during its life cycle sugarcane requires a huge water amount with an annual rainfall of at least 1500–2000mm (Food and Agriculture Organization of the United Nations, <http://www.fao.org>). Therefore, water deficit is often the main factor constraining sugarcane production.

In sugarcane production, drought stress frequently occurs during early growth stage at tillering and early grand growth phase. During this stage, macronutrients especially nitrogen are often fertilized to promote sugarcane growth. Drought stress, therefore, at first reduces nitrogen uptake (T. Silva, Cazetta, Carlin, & Telles, 2017), then affects the assimilation and remobilization processes of nitrogen by restricting enzyme activity e.g. nitrate reductase (Abayomi, 2001), and results in the decline of nitrogen use

efficiency (NUE). Previous studies found that there were the positive associations between NUE traits with sugar yield and cane yield (Acreche, 2017). Similarly, the improvements in plant growth and biomass production in sugarcane genotypes incorporate with NUE traits (Calif & Edgcombe, 2015). Moreover, Ranjith and Meinzer (1997) found that NUE was significantly higher in drought-resistant genotype than in the susceptible genotype. It raised a hypothesis that higher NUE could improve drought tolerant ability in sugarcane. In fact, it was demonstrated by a positive correlation between NUE and drought tolerant ability in sugarcane NiF8 variety (Dinh, Watanabe, Takaragawa, Nakabaru, & Kawamitsu, 2017). However, whether there is the same relationship between NUE and drought tolerant ability in terms of various sugarcane varieties. This study was conducted to evaluate growth, biomass performance, NUE and drought tolerant ability of different sugarcane varieties under drought stress at early growth stage; and to get a better understanding about the relationship between NUE and drought tolerant ability in sugarcane.

Materials and methods

Experimental design

The pot experiment was conducted under glasshouse condition at the University of the Ryukyus, Okinawa, Japan (26°25'N, 127°45'E; altitude 126 m) from May to September 2017.

The experiment was divided into two blocks:

- (i) Block 1 – five commercial sugarcane varieties, including NiF3, Ni9, Ni17, Ni21 and Ni22 (Table 1) at well-watered (WW) condition were assigned in a randomized complete block design with three replications;
- (ii) Block 2 – a split-plot design was used with five replications. Two soil water regimes including WW and water stress (DS) for 60 days from 60 days after transplanting (DAT) to 120DAT were assigned in the main plots. The five above varieties were designed in the subplots.

The 2-month-old seedlings were transplanted into Wagner pots (1/2000 a) filled with 8.5 kg substrate of Shimajiri Mahji red soil: sea sand: peat moss (1:1:1, v v⁻¹) at the gravimetric soil moisture content of approximately 5.9 %. The experimental pots were arranged in 40 × 90 cm of distance between each pot and pot row. During the experimental period, all tillers were removed immediately after emergence. Plant in each pot was fertilized weekly by replacing irrigation with 500 mL of the modified Hoagland's nutrient solution with a composition of 6mM

Ca(NO₃)₂·4H₂O, 4 mM KNO₃, 2 mM KH₂PO₄, 2 mM MgSO₄·7H₂O, 25 μM H₃BO₃, 10 μM MnSO₄·5H₂O, 2 μM ZnSO₄·7H₂O, 0.5 μM CuSO₄·5H₂O and 0.1 mM C₁₀H₁₂FeN₂NaO₈·3H₂O (Fe-EDTA).

Water management

As soon as after transplanting, water was added to increase soil moisture to field capacity at volume soil moisture of 30% which was monitored by volume water sensors (5TE soil moisture and temperature, Decagon Devices Inc., USA) which were set up at 10 cm of depth. For WW treatment, irrigation was done with full water loss that was calculated daily by a balance (A&D, FG-30KBM) until the end of the experimental period. For water stress treatment, water application was practiced as same as the WW treatment until 60 DAT. After that, water was applied by just 50% of water loss until soil moisture content reaching to 15% of volume soil moisture (equivalent to 1/3 available water), then by full water loss of this treatment until the end of the experiment.

Data collections

From 28DAT, growth parameters including total leaves number and plant height of each variety in both water treatments were measured at a 2-week interval.

In the block 1: One day before starting water stress treatment, the first fully expanded leaves of the sample plants were taken to determine photosynthetic parameters i.e. photosynthetic rate (A_{max}), stomatal conductance and transpiration rate using a portable photosynthesis system (LI-6400, LI-COR, Lincoln, Nebraska, USA) equipped with a 2 × 3 cm LED chamber between 0900 and 1500 at a PFD of 2000 μmol m⁻² s⁻¹, leaf temperature of 31 ± 2°C, CO₂ concentration of 400 ± 5 μmol mol⁻¹. After photosynthesis measurement, SPAD values were recorded at the same positions using a SPAD meter (SPAD-502, Minolta, Japan). At 60DAT, all plants were cut to determine leaf area and dry matter accumulation. Green leaves were cut to determine leaf area using a leaf area meter (LI-3100, Li-COR, Lincoln, Nebraska, USA). After that, leaves, stalk (after squeezing) and root (after cleaning by tap water) were separately oven-dried at 80°C for 48 h to determine partial dry weights.

In the block 2: From 2 days before finishing the experiment, the first fully expanded leaves of three sample plants of each variety were taken to determine photosynthetic parameters and SPAD values. After that, the measured leaves were cut to determine the leaf area and oven-dried at 80°C for 48 h to determine dry weight. At 120DAT when drought stress treatment was

Table 1. List of investigated sugarcane varieties.

Varieties	Characteristics and suggested regions for cultivation			
	Leaf blade (Length/width)	Stalk type	Yield potentiality	Suggested regions
NiF3	Short/wide	Long, thick, numbers: small	High	Tanegashima island
Ni9	Long/medium	Long, slightly thin, numbers: large	High	All areas in Okinawa Prefecture
Ni17	Medium/slight wide	Medium, thick, numbers: small	High	Middle to the southern part of Okinawa mainland, Kume island & Amami region
Ni21	Medium/slight wide	Slight long & thick, numbers: small	High	Kume island
Ni22	Medium/medium	Slight long & thick, numbers: large	High	Tanegashima & Amami region

Source: Takagi, Sato, and Matsuoka (2005); Alic (n.d) (<https://www.alic.go.jp>)

completed, all plants were cut separately into leaves, stalk, and root. The stalk (after squeezing), leaves (after scanning leaf area) and root (after cleaning by tap water) were dried at 80°C for 48 h to determine partial dry weights. After that, the first leaf and other leaves, stalk and the root of the sample plants were separately ground by vibrating sample mill (TI-100, CMT, Tokyo, Japan). Then, these parts were well-mixed again by the ratio of partial weight. 25 mg of each first leaf and mixture were taken to determine nitrogen content using an N/C analyzer (NC-90A, Shimadzu, Japan).

Calculation for nitrogen use efficiency traits and drought tolerant indexes

After determining partial dry weight, the aboveground dry weight was calculated by the sum of the stalk and leaves dry weight. Total dry weight was calculated by the sum of the aboveground and root dry weight.

Biomass nitrogen use efficiency (NUE_b) was calculated by following formula:

NUE_b ($g\ g^{-1}$) = total dry weight/total nitrogen applied amount;

The measured first leaf nitrogen content was used to calculate specific leaf nitrogen content (NL) and photosynthetic nitrogen use efficiency (PNUE) as following:

NL ($g\ m^{-2}$) = measured nitrogen content \times leaf dry weight/leaf area;

$PNUE$ ($\mu mol\ s^{-1}\ g^{-1}$) = A_{max}/NL ;

The mixture nitrogen content (TN) was used to calculate total nitrogen uptake (TNU) and nitrogen utilization efficiency (NUE_t) by the following formulas:

TNU (g) = TN \times total dry weight;

NUE_t ($g\ g^{-1}$) = total dry weight/TNU;

Drought tolerant index (DTI) was determined as following:

DTI = dry weight under stress condition/dry weight under WW conditions.

Statistical analysis

The data were subjected to analysis of variance according to a split-plot and randomized complete block design using Statistix 8.0 package. Turkey test was used to compare the means. Correlation coefficients among NUE traits and DTI were calculated to assess the relationships.

Results

Meteorological conditions and volume soil moisture in the experimental site were shown in Figure 1. During the experimental time, the daily average air temperature, air humidity and solar radiation in glass house ranged from 22.0 to 33.6°C, 59.6 to 91.6%, and from 9.2 to 180.6 $W\ m^{-2}$, respectively. Soil moisture content of WW treatment changed from approximately 30% at the beginning to around 25% at the end of the experiment. The reduction in soil moisture of this treatment may be because along with stalk elongating water moved from soil to store in the stalk that leads to a shortage of water in the soil. Meanwhile, in stress treatment, soil moisture content fluctuated around 30% until 60DAT (before stress treatment), then reduced steadily during the first 20 days after starting treatment, before changing around 15% from 80 to 120DAT.

From Figure 2, there were obvious differences in total leaves number and plant height of sugarcane varieties from 56DAT. NIF3 showed the highest total leaves number and plant height in comparison with the counterparts under

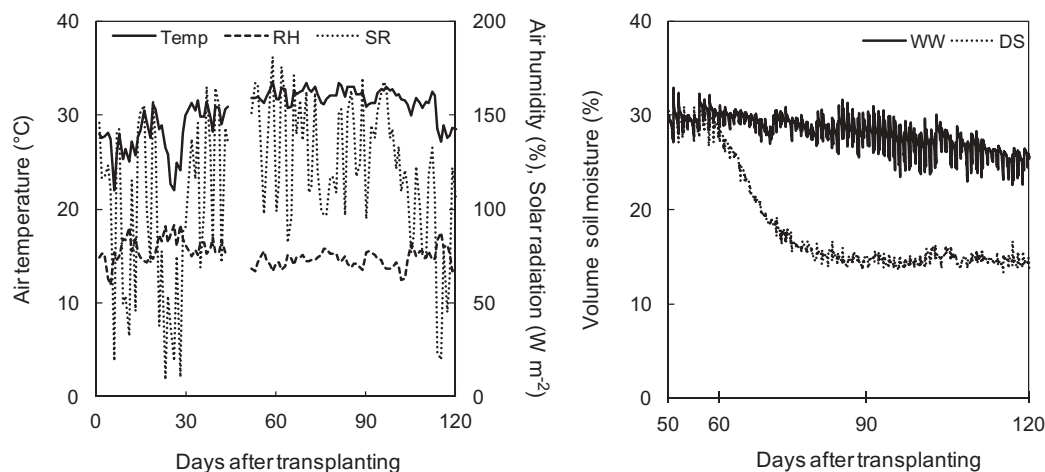


Figure 1. Weather conditions and soil moisture content during the experimental period Temp, RH and SR: daily average air temperature, air humidity and solar radiation; WW: well-watered; DS: water stress.

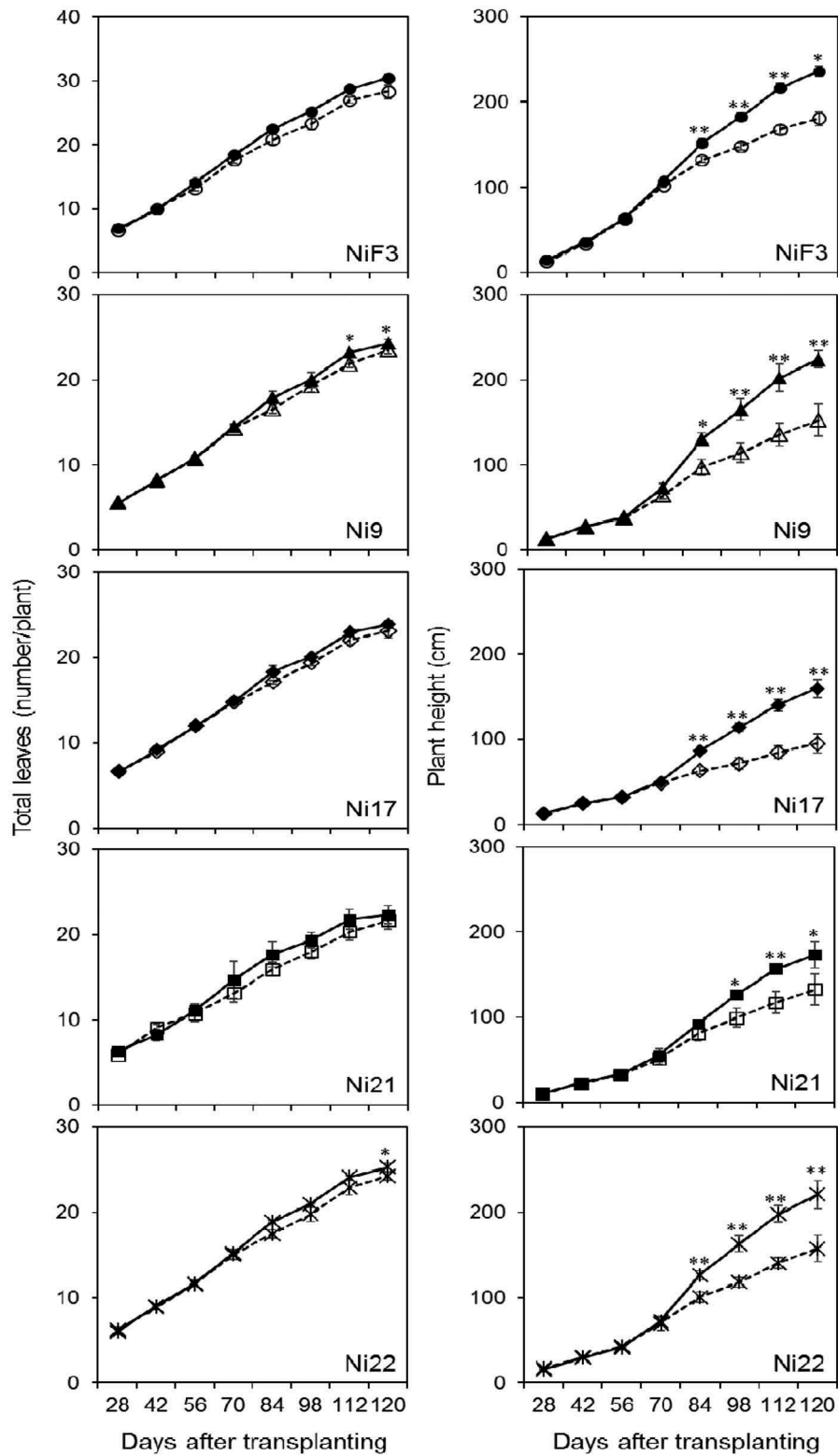


Figure 2. Total leaves number and plant height growth of sugarcane varieties under WW (filled symbols, solid line) and water stress conditions (non-filled symbols, dot line), respectively. * and ** mean non-significant between WW and water stress treatment, significant at $p < 0.05$ and $p < 0.01$, respectively.

both WW and stress conditions. Meanwhile, Ni21 and Ni17 showed the lowest values for total leaves number and plant height, respectively. The effects of drought stress on total

leaves number and plant height were observed clearly from 84DAT with slower growth rates under drought stress treatment comparing to those under WW condition.

The effects of different sugarcane varieties and water regimes on A_{max} and growth parameters were shown in Figure 3. At 60DAT, A_{max} of sugarcane varieties ranged from 38.2 to 45.2 $\mu\text{mol m}^{-2} \text{s}^{-1}$. A_{max} of Ni21 was lowest and significantly lower than those of NiF3, Ni9 and Ni17 (Figure 3(a)). However, at 120DAT, there were no significant differences in A_{max} of varieties under both WW and stress conditions (Figure 3(d)). At this time, drought stress affected A_{max} with reduction of 19.4–26.1%. Ni21 had the highest reduction rate, whereas NiF3 did the lowest one, but the difference was not remarkable

among target varieties (data not shown). Different varieties had significant differential effects on leaf area and plant height at both 60 and 120DAT. In fact, at 60DAT, leaf area of Ni17 was highest and significantly higher than that of Ni9 and Ni21. Ni17 also had highest leaf area at 120DAT and significantly higher than Ni9 did under the WW condition and all other varieties did under stress condition. Ni21 had lowest leaf area which followed by Ni9 at 60DAT. However, at the later period, Ni21 grew faster and had higher leaf area than Ni9 under both water conditions (Figure 3(b,e)). At

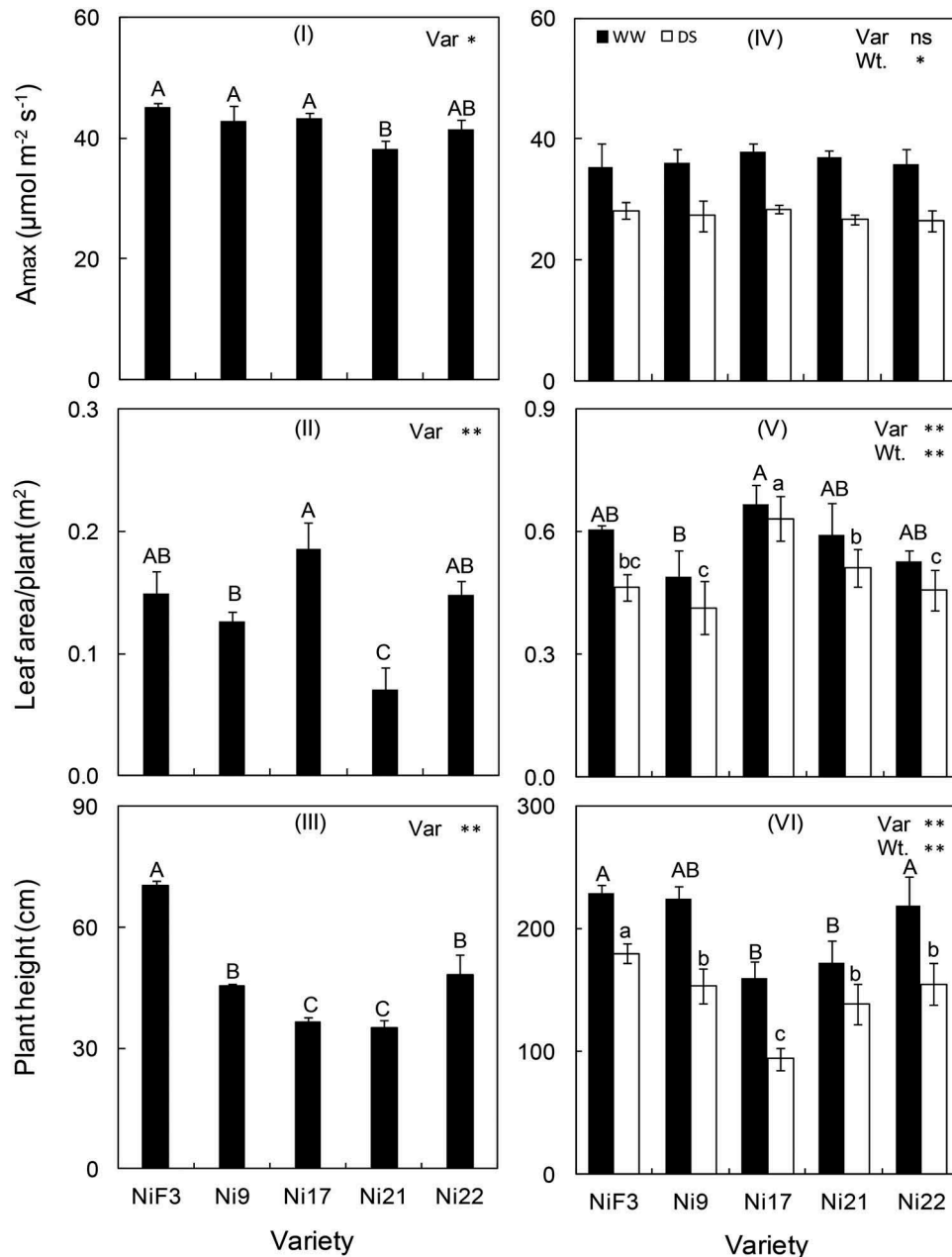


Figure 3. Potential photosynthetic rate (A_{max}), leaf area and plant height of sugarcane varieties under different water regimes at 60DAT (a, b, c) and 120DAT (d, e, f), respectively. WW: well-watered; DS: water stress; Var: variety; Wt.: water regime. Different capital and lowercase letters show significance among varieties at well-watered and water stress conditions at $p < 0.05$ by Turkey, respectively. ns, * and ** mean non-significant, significant at $p < 0.05$ and $p < 0.01$, respectively.

60DAT, NiF3 had the highest plant height, whereas Ni17 and Ni21 did the lowest ones. At 120DAT, NiF3 also did the highest plant height, significantly higher than Ni17 and Ni21 did under WW and all other varieties under drought stress condition. Meanwhile, Ni17 and Ni21 did the lowest plant height under both conditions (Figure 3 (c,f)). Drought stress reduced significantly leaf area and plant height of all target varieties with the reduction rate of 6.9–21.3% and 19.4–41.2%, respectively (Figure 3 (e,f)). Ni17 had the lowest reduction rate in leaf area but highest in plant height. On the contrary, NiF3 had the highest reduction rate in leaf area but lowest in plant height.

As can be seen from Figure 4, various varieties and water regimes had different values for total and partial dry weights. Actually, the aboveground (including leaves and stalk), root and total dry weight of sugarcane varieties under drought stress condition were significantly lower than those under WW condition (Figure 4(d–f)). Ni21 and Ni9 showed the lowest values for dry weights than others at 60DAT (Figure 4(a–c)). They also did the lowest values for all traits under both water conditions at the later period (Figure 4(d–f)). At 120DAT, NiF3 had the highest aboveground dry weight, root as well as total dry weight, which followed by Ni17 and Ni22 at both WW and stress conditions. NiF3 and Ni17 also had the lowest reduction rates of total dry weight under the effect of drought stress in comparison with other varieties. In addition, the highest value and lowest reduction rate for stalk dry weight were shown in NiF3 and for leaf dry weight in Ni17, respectively (Figure 4(d–f)).

Drought stress significantly reduced PNUE, NUE_t and NUE_b of sugarcane varieties (Table 2) with the reduction rate of from 17.1 to 31.0%, 19.8 to 29.3% and from 22.8 to 31.4%, respectively. However, it did not affect NL and TNU, whereas TN significantly increased under the effect of drought stress. Sugarcane varieties had different NL, TN, PNUE, NUE_t , NUE_b and DTI, where NiF3 and Ni17 had the lowest values for NL and TN, but highest for NUE parameters and DTI. In fact, PNUE of Ni17 was significantly higher than all other varieties under both water conditions (excepting for NiF3 under drought stress condition). NiF3 had highest NUE_t and NUE_b under both water conditions, but NUE_t was not significantly higher than Ni17 under drought stress condition. NiF3 had highest DTI which noticeable higher than other varieties excepting for Ni17 (higher but not significant). NiF3 also had the lowest reduction rate of PNUE, NUE_t and NUE_b .

The interactions between water regimes and varieties were not significant for nitrogen-related traits (Table 2), A_{max} growth and dry matter parameters (data not shown). It indicated that variety with high

potential for target traits under WW conditions performed well under drought stress condition at the early growth stage.

DTI had positive significant correlations with PNUE ($r = 0.66^{**}$), NUE_t ($r = 0.58^*$) and NUE_b ($r = 0.76^{**}$) (Figure 5). The correlations of partial DTIs and NUEs also showed positive relationships (Figure 6). Ni17 had highest partial DTI and NUE by leaves dry weight, whereas NiF3 showed the highest partial DTIs and NUEs by the stalk and aboveground dry weight. Partial DTIs had contribution to total dry weight DTI with strong positive correlation coefficients of DTI with DTI_stalk ($r = 0.80^{**}$) and DTI_above ($r = 0.89^{**}$) (Table 3).

Discussion

The closure of stomata, the gateway of CO_2 exchange between plant leaf and its living environment, when plant subjects to water stress to restrict water loss is also the reason for the reduction of the photosynthetic rate by the shortage of substrate supporting photosynthetic activity. Lacking energy and materials from photosynthesis leads to restricting cell division and elongation processes resulting in the reduction of growth namely in plant height, green leaves number and dimension. In this study, the growth and photosynthesis of sugarcane varieties significantly decreased when they subjected to water stress. It concurs with many previous studies in the negative effects of drought stress on photosynthetic rate (Barbosa et al., 2015; Dinh et al., 2017; Graça et al., 2010; Ribeiro et al., 2013), plant height and plant elongating rate (Barbosa et al., 2015; Dinh et al., 2017; Ethan, Olagoke, & Yunusa, 2016; Zhao, Glaz, & Comstock, 2010), leaf number and leaf area (Barbosa et al., 2015; Dinh et al., 2017; Robertson, Inman-Bamber, Muchow, & Wood, 1999). The decrease of source and sink (photosynthesis following by vegetative tissue growth) leads to declining dry matter accumulations in both partial and whole plant. Our results are in line with the previous studies in the reductions of leaves, stalk, root and total dry weights under effect of drought stress at early growth stage (Barbosa et al., 2015; Dinh et al., 2017; Robertson et al., 1999; Wagih, Ala, & Musa, 2003; Zhao et al., 2010).

Genetic variation in photosynthetic rate, leaf area; tops, root and total biomass was found in sugarcane varieties (Basnayake, Jackson, Inman-Bamber, & Lakshmanan, 2012; Jackson et al., 2016; Li et al., 2017; Luo et al., 2014; Ramesh, 2000). In our study, significant variations were found in growth and dry matter parameters at both 60 and 120DAT, whereas the difference in A_{max} just at 60DAT among Ni21 with NiF3, Ni9, and

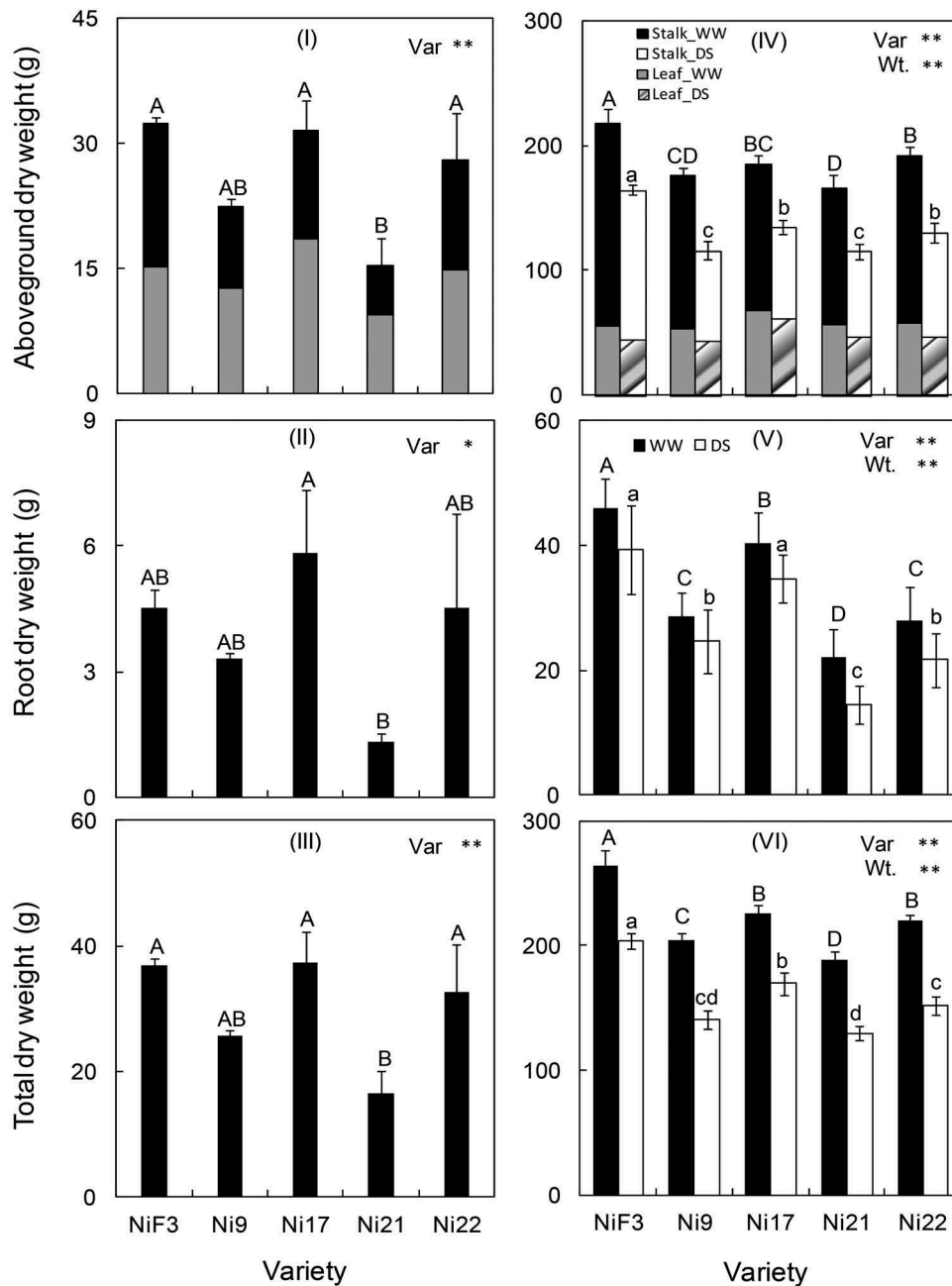


Figure 4. Aboveground (leaf and stalk), root and total dry weight of sugarcane varieties under different water regimes at 60DAT (a, b, c) and 120DAT (d, e, f), respectively. WW: well-watered; DS: water stress; Var: variety; Wt.: water regime. Different capital and lowercase letters show significance among varieties at well-watered and water stress conditions at $p < 0.05$ by Turkey, respectively. ns, * and ** mean non-significant, significant at $p < 0.05$ and $p < 0.01$, respectively.

Ni17 at 60DAT. At 120DAT, there were no significant differences in A_{max} under both drought stress and WW condition. The significant difference did not find in A_{max} , but in partial and total dry matters that made the differences in photosynthetic efficiency of investigated varieties. Previous studies reported that drought tolerant varieties have better performance as well as lower reduction rate of growth and biomass parameters in comparison with drought sensitive ones (Begum &

Islam, 2012; Hemaprabha, Swapna, Lavanya, Sajitha, & Venkataramana, 2013; M. Silva, Jifon, Da Silva, & Sharma, 2007; Wagih et al., 2003). In this study, NiF3 and Ni17 presented better performance under both water conditions with the lower reduction rate of A_{max} , leaf area, plant height as well as dry weight than other varieties. This could be explained by these two varieties had better drought tolerant ability (DTI = 0.77 and 0.75, respectively) than other ones (DTI = 0.69).

Table 2. Specific leaf nitrogen content (NL), total nitrogen content (TN), TNU, PNUE, biomass nitrogen utilization efficiency (NUE_t) and biomass nitrogen use efficiency (NUE_b) and total dry matter-based DTI of sugarcane varieties at 120DAT.

Variety	Water levels	NL (g m ⁻²)	TN (%)	TNU (g)	PNUE (μmol s ⁻¹ g ⁻¹)	NUE _t (g g ⁻¹)	NUE _b (g g ⁻¹)	DTI	
NiF3	Well-watered	0.93 ^b	0.37 ^c	1.01	37.0 ^b	266.6 ^a	152.2 ^a	–	
Ni9		1.03 ^{ab}	0.52 ^a	1.04	34.9 ^{bc}	194.1 ^c	118.0 ^c	–	
Ni17		0.80 ^c	0.45 ^b	1.02	46.2 ^a	225.0 ^b	130.1 ^b	–	
Ni21		1.00 ^{ab}	0.51 ^a	0.98	37.8 ^b	195.11 ^c	108.5 ^d	–	
Ni22		1.10 ^a	0.49 ^{ab}	1.08	32.2 ^c	203.4 ^{bc}	126.9 ^b	–	
NiF3		Water stress	0.93 ^{ab}	0.48 ^c	0.97	30.7 ^{ab}	208.9 ^a	117.5 ^a	0.77 ^a
Ni9			1.10 ^a	0.64 ^{ab}	0.93	25.2 ^b	155.7 ^{ab}	81.0 ^{cd}	0.69 ^b
Ni17			0.83 ^b	0.58 ^{bc}	0.99	34.3 ^a	172.1 ^{ab}	97.6 ^b	0.75 ^{ab}
Ni21			1.00 ^{ab}	0.73 ^a	0.92	26.1 ^b	138.0 ^b	74.7 ^d	0.69 ^b
Ni22			1.07 ^a	0.66 ^{ab}	1.02	25.2 ^b	152.4 ^b	87.5 ^c	0.69 ^b
Ni22			1.07 ^a	0.66 ^{ab}	1.02	25.2 ^b	152.4 ^b	87.5 ^c	0.69 ^b
<i>Source of variance</i>									
Variety (Var.)		**	**	ns	**	**	**	**	
Water level (Wt.)		ns	**	ns	**	**	*	–	
Var.*Wt.		ns	ns	ns	ns	ns	ns	–	

ns, * and ** mean non-significant, significant at $p < 0.05$ and $p < 0.01$, respectively. DAT: days after transplanting. Different small letters in the same column show significance between sugarcane varieties at the same water levels at $p < 0.05$ by Turkey.

Drought stress increased significantly TN but did not affect NL (Table 2). Dinh et al. (2017) also found the same results where NL seemed to reduce at lower nitrogen application levels but increased at higher levels when plant subjected to water stress. Similarly, Ludlow, Ferraris, and Chapman (1991) found the reduction of NL in three of six investigated cultivars, but NL did not change in two other and even increased in var. Q50. Silva et al. (2017) reported the uptake of nitrogen was reduced significantly under the effect of water stress. In this study, drought stress reduced TNU of all varieties, but not significantly. Water shortage might reduce dissolved nitrogen ability (in the form of urea which was applied just 10 days before plant subjected to water stress) which leads to reducing the amount of nitrogen uptake in Silva et al. (2017)'s experiment. Meanwhile, in this study, the dissolved nitrogen was applied weekly even during water stress period. Therefore, drought stress did not have a clear effect on TNU. However, it reduced leaf area and plant height, as the result, nitrogen was concentrated with higher density being the reason for the higher concentration in plant tissues.

Under irrigated conditions, the pieces of evidence of differences in NUE traits, in sugarcane varieties were reported by Schumann, Meyer, and Nair (1998), Ranjith and Meinzer (1997), Robison et al. (2009) and Robison, Schmidt, and Lakshmanan (2014). This study also showed the difference in NUE parameters among sugarcane varieties in both drought stress and well-water conditions. In the plant, after being uptake, nitrogen is used to create new organs throughout assimilation and remobilization processes by reductase and synthetase enzymes i.e. nitrate reductase or glutamine synthetase (Lattanzi, Schnyder, & Thornton, 2005). The reduction of NUE through nitrate reductase activity

when sugarcane subjected to drought stress and the NUE variation between selected sugarcane cultivars were reported (Abayomi, 2001). In this study, although the same amount of nitrogen uptake, differences in the accumulated dry matter between water regimes and among sugarcane varieties showed significant differences NUE were contributed from nitrogen utilization efficiency rather than nitrogen uptake efficiency.

The positive correlation among DTI and NUE parameters (Figure 5) suggested that higher NUEs, especially NUE_b, could support higher drought tolerant ability in sugarcane. It agreed with Ranjith and Meinzer (1997) that dry matter-based NUE of drought-resistant genotype (H69-8235) was always significantly higher than that of the susceptible genotype (H65-7052). It is interesting that NiF3 and Ni17 had higher NUEs as well as total dry matter DTI than varieties did, but they showed different expressions in partial DTIs and NUEs. Whilst NiF3 showed better aboveground and stalk NUEs and DTIs, Ni17 had higher leaves NUE and DTI than remainders. In fact, by observation Ni17 had clear different style with shorter stalk but larger leaf area than others. Trying to evaluate the contributions of partial DTIs to total DTI, we found that aboveground contributed more than root did, and stalk had the larger contribution than leaf did. From this result, we can suggest that DTI for aboveground could be used as a replacement for total dry weight in evaluating drought-tolerant ability. Moreover, DTI for stalk should be used along with aboveground/total dry weight as an extra evaluation.

In this study, NiF3 (high remained stalk weight) seemed to be better for the tolerant ability for drought stress at early growth stage than other varieties. In Japan, in the actual field, drought tolerant ability of sugarcane varieties is often evaluated by the observation based on

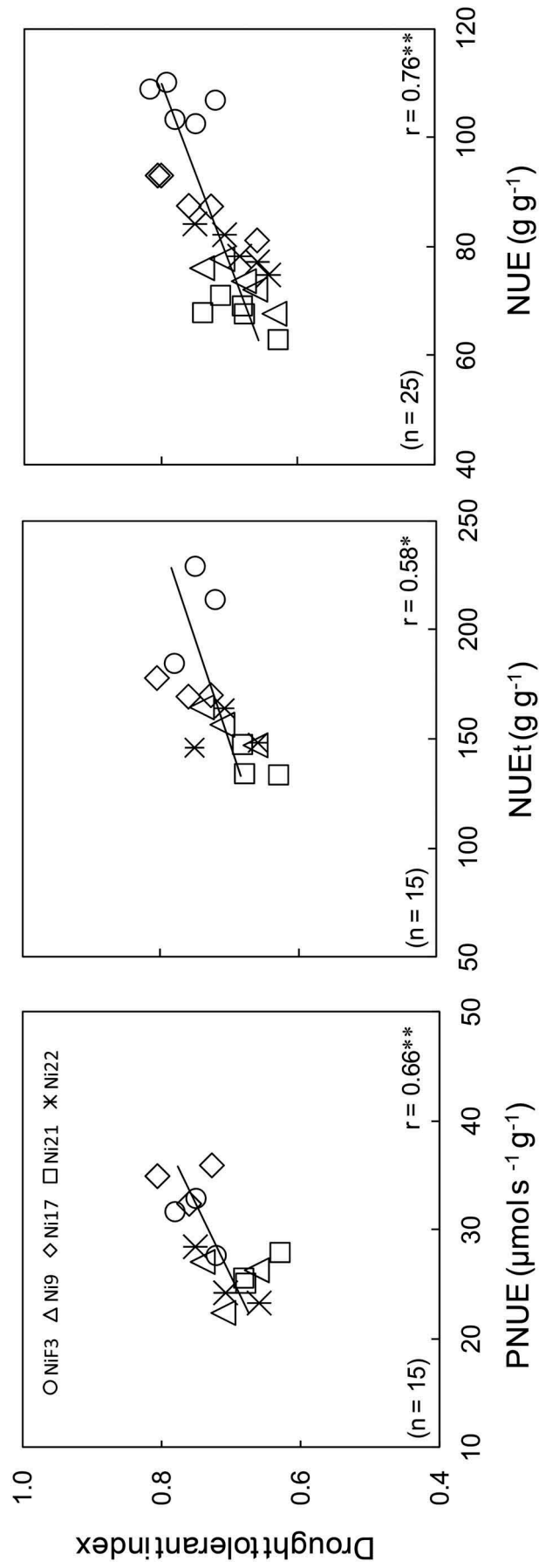


Figure 5. Correlations among total dry matter-based DTI with photosynthetic nitrogen efficiency, biomass nitrogen utilization efficiency (NUE_t) and biomass nitrogen use efficiency (NUE_b) of sugarcane varieties at 120DAT * and ** mean significant at $p < 0.05$ and $p < 0.01$, respectively.

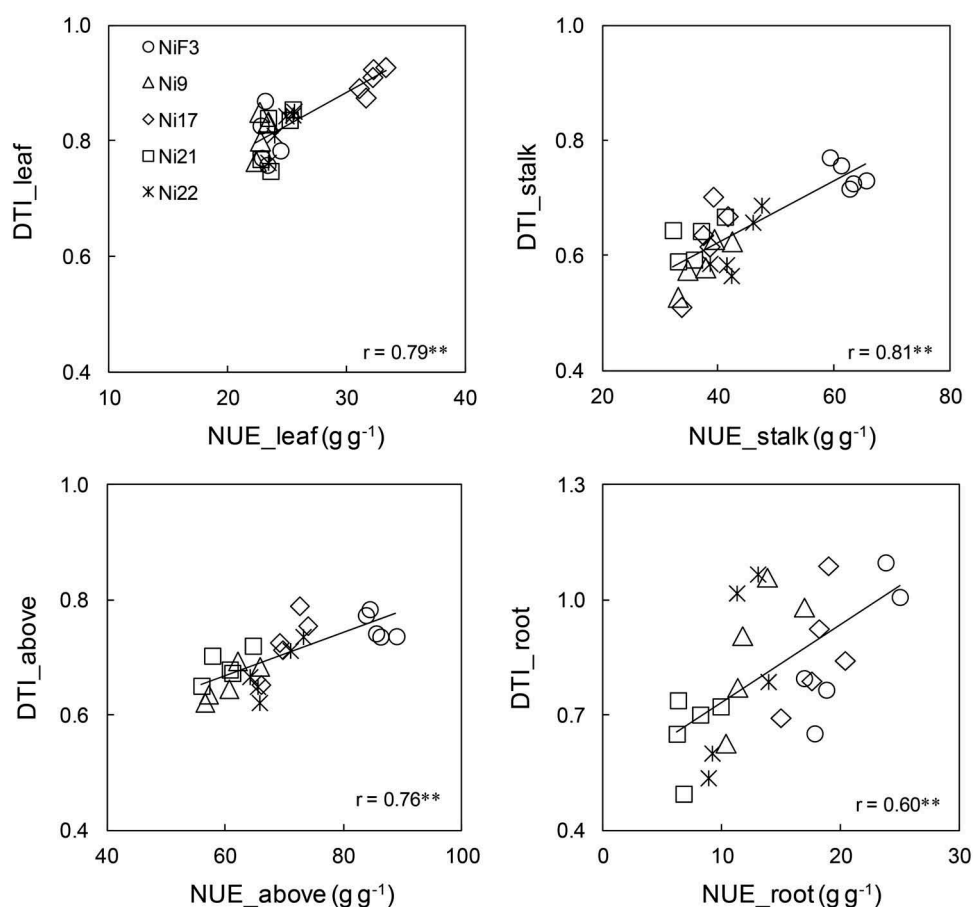


Figure 6. Correlations among partial DTIs and biomass nitrogen efficiencies (NUE) for aboveground parts (including leaves and stalk) and underground part (root) of sugarcane varieties at 120DAT * and ** mean significant at $p < 0.05$ and $p < 0.01$, respectively. DTI_..., NUE_...- partial drought tolerant index and nitrogen use efficiency for root, leaf, stalk and aboveground, respectively.

Table 3. Contributions of partial DTIs to total dry matter-based DTI.

Source	DTI
DTI_root	0.59**
DTI_leaf	0.38 ^{ns}
DTI_stalk	0.80**
DTI_aboveground	0.89**

ns and ** mean non-significant, significant at $p < 0.01$. DTI_... partial drought tolerant index for root, leaf, stalk and aboveground.

the leaf senescence (Plant Variety Protection [PVP, n.d.], <http://www.hinshu2.maff.go.jp>) or the reduction of stalk length after drought stress period. By this evaluation, NiF3 was also considered as strong tolerant variety (National Agriculture and Food Research Organization [NARO, n.d.], <http://www.gene.affrc.go.jp>). Ni9, Ni21 and Ni22, recently, are considered as drought tolerant varieties, whereas Ni17 is considered as a little weak tolerant (Okinawa Prefectural Government, Department of Agriculture, Forestry and Fishery, 2015). However, in this study, Ni17 seemed to be better tolerant to drought stress (not significant) than Ni9, Ni21 and Ni22 (Table 2). It is quite difficult to compare our study to actual field

evaluation for drought tolerance, because of several differences. In this study, we tried to evaluate under the same soil moisture condition, whereas under actual field soil moisture may be different because of different water consumption from varieties. Moreover, in this study, we just concerned for a drought tolerant ability at the early growth stage, meanwhile in the actual field, drought stress may occur at different growth stages because growing condition is under rain-fed conditions. For instance, in another reports, Ni9 was considered as a relative (Matsuoka, 2006) or a little weak tolerance (Alic, n.d) to drought stress. Similar to Ni17, NiF8 is often considered as a little weak (Okinawa Prefectural Government, Department of Agriculture, Forestry and Fishery, 2015) or a medium type for drought stress (PVP, n.d.F), but in NARO's report, it was also considered as a tolerant variety (NARO, n.d.). The limited environment under pot condition (small root volume) with only one kept stalk may affect by stalk weight and stalk numbers. Although Ehara et al. (1994) reported that no significant differences between stem weight and stem number type in dry matter yield, but this report was

under field conditions where all characteristic of varieties was shown. In this experiment, stalk weight type seems to prevail over stalk number type in dry matter, NUE as well as DTI, but Ni9 and Ni22 (stalk number type) somewhat showed no differences for these parameters with Ni17, even higher than Ni21 (stalk weight type). Therefore, to confirm our suggestion on drought tolerant ability of sugarcane varieties, the further demonstrations under non-limited conditions at the field scale should be practiced in later studies.

Disclosure statement

No potential conflict of interest was reported by the authors.

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