STUDY ON NITROGEN USE EFFICIENCY OF SUGARCANE (*Saccharum* spp.) UNDER DROUGHT STRESS CONDITIONS

水ストレス条件下のサトウキビの窒素利用効率に関する研究

Doctoral Thesis

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SUMMARY

Drought stress, which frequently occurs at early growth stage, may be a reason for the reductions of nitrogen (N) uptake and N use efficiency (NUE), resulting in low yield and sugar quality in sugarcane. For this reason, it was hypothesized that improvement of NUE could help sugarcane confront to drought stress or selecting varieties with higher NUE could be a strategy for sugarcane production under drought stress conditions. This study investigated the relationship between NUE and drought tolerant ability under different levels of N application with various varieties in order to provide beneficial information for the current breeding program aiming at better drought tolerant ability at early growth stage in sugarcane.

The responses to water stress and N application in physiological and agronomical characteristics and the relationship between NUE and drought tolerant ability of a commercial variety, NiF8, were investigated in Chapter 2. The results showed that the drought reduced the growth, biomass and NUE traits. Applying N supported better physiological and agronomical performance, but increasing N did not result in higher growth and NUE, especially under the drought stress conditions. The strong positive correlation coefficients between NUE traits and drought tolerance index (DTI) may suggest that higher NUE traits could help the plant have a better ability to tolerate drought stress.

The growth, NUE, and drought tolerant ability of different sugarcane varieties were evaluated in Chapter 3 to get a better understanding about the relationship between NUE and drought tolerant ability in sugarcane. An experiment was conducted under a glasshouse condition with five sugarcane varieties under different water regimes. The results showed the drought reduced the photosynthetic rate, growth parameters and NUE traits. Varietal differences were found in all growth- and NUE-related traits and DTI. The positive correlations between the NUE traits and DTI also suggested that higher NUEs could support a better tolerant ability to drought stress.

In Chapter 4, the daily changes in soil moisture content and photosynthetic response were observed to point out the critical soil moisture value that will be helpful information for irrigation management in sugarcane. The results showed the photosynthesis changed in parallel with changing of soil moisture content. Photosynthesis could be a useful indicator to determine the time to start irrigation. Irrigation should start at a soil volume moisture content of 15% or a pF of 2.8 to avoid any reduction of photosynthesis.

From Chapter 2 and Chapter 3, it could be concluded that the NUE traits should be an added tool along with yield components for screening drought tolerant sugarcane varieties in the future breeding. In Chapter 5, screening of the commercial sugarcane varieties by NUE traits was conducted under a rain-fed condition. The results showed that the drought stress caused reductions in plant height and SPAD, but they were not statistically significant. Genetic variations in growth, yield components, and biomass traits were found among investigated varieties. The positive associations between NUE traits and total biomass production suggested that higher NUE traits could support better growth performance of sugarcane under rain-fed conditions. From this study, NiF3, NiF8, and Ni27 showed the best performance with the highest growth, yield components, and biomass parameters as well as NUE.

In conclusion, higher drought tolerant ability in sugarcane resulted from better use of N source and NUE traits could be key tools to screen drought tolerant sugarcane varieties to water stress at early growth stage. NiF3, NiF8 and Ni27 could be introduced as promising varieties in terms of NUE performance and drought tolerant ability and thus be used as reference varieties or crossing materials in the future breeding program.

SUMMARY IN JAPANESES

サトウキビの初期成育期に頻繁に生じる土壌乾燥ストレスは窒素吸収および窒素利用効率（NUE）の低下の要因のひとつであり，結果として収量および品質の低下を引き起こす．こういった理由から，NUEの向上が乾燥ストレス下のサトウキビの生育に不可欠であり，高NUE品種の選択が乾燥条件下でのサトウキビ生産の鍵となると仮説を立て，その検証を行った．本研究ではサトウキビ初期成育期の耐乾性の向上を目的とし，育種プログラムに有益な情報を提供するため，複数品種に異なる窒素量を与えた処理を行い，NUEと耐乾性の関係性を調査した.

第2章では，主要品種であるNiF8を用いて，生理学的パラメーター，収量関連形質における水ストレスと窒素処理への反応およびNUEと耐乾性の関連性について調査した．成育，乾物生産およびNUE関連形質は乾燥ストレスにより阻害された．これらの形質は窒素施用区で高くなる傾向がみられたものの，特に乾燥ストレス下では窒素施用量の増加による影響は認められなかった．NUE関連形質と耐乾性指数（DTI）との間には高い正の相関関係が確認されたことから，NUE関連形質は耐乾性と密接に関係していると考えられた.

次に第3章では，NUEと耐乾性の関係をより深く理解するため，5品種を用いて灌水条件を変化させたポット試験を行い，成育，NUEおよび耐乾性の評価を行った．乾燥ストレスを与えた結果，光合成速度，成育およびNUE関連形質の低下がみられ，さらに成育，NUE関連形質およびDTIには品種間差異が認められた．本試験においても，NUE形質とDTIの間には強い関係性が認められ，耐乾性の改善にはNUEの向上が必要であることが示唆された.

第4章では，サトウキビの灌水管理において重要な土壌含水率の閾値を明らかにするため，土壌含水率と光合成速度の日変化の観測を行った．土壌含水率の変化に光合成は敏感に反応したことから，灌水方法の決定に利用可能な指標であると考えられた．本試験結果から，光合成速度の低下を抑制するために土壌含水率が15%以下，もしくはpF値が2.8以上となったときに灌水を行う必要があると考えられた.

第2章および第3章から，育種分野における耐乾性品種のスクリーニングを効率化するための手段として，収量構成要素に加えNUE関連形質を利用する必要がある

と結論付けられた．そこで第5章では，天水条件下で主要品種の栽培を行い，NUE形質を用いたスクリーニングに試供した．統計的に有意ではなかったが，乾燥条件下で仮茎長およびSPAD値は減少した．また，成育，収量構成要素および乾物生産において品種間差異が確認された．NUE形質と総乾物生産量との間には密接な関係が認められたことから，天水条件下での成育の維持にNUE形質の向上が必要であることが示唆された．NUEに加え，成育および収量が高かったことから，本試験条件下ではNiF3，NiF8，Ni27が有望な品種であることが明らかになった．

本研究より，サトウキビにおける耐乾性は窒素利用と密接に関連しており，したがってNUE関連形質の利用は初期成育期の耐乾性品種の選抜に有効な手段となると結論付けられた．NUEおよび耐乾性の観点からNiF3，NiF8，Ni27が有望な品種であると評価されたことから，今後の育種プログラムではこれらの品種を耐乾性の評価基準や育種素材として利用可能であることが示された．

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CHAPTER I

GENERAL INTRODUCTION

1.1. Worldwide sugarcane production

Sugarcane, an essential cash crop, derives approximately 80% of the global sugar production (International Sugar Organization, 2017). It is also considered as an important alternative fuel source which 40 - 50% of the world ethanol production is based on sugarcane (Zuurbier and Vooren, 2008). Moreover, sugarcane plant is a perennial grass with the huge green biomass which creates from the tall and leafy stalks, and that could be used as a forage crop for livestock (Feedipedia, http://www.feedipedia.org).

Nowadays, sugarcane has been grown in over 100 countries in the world. Since the year 2000, the world sugarcane production has been increasing in the harvested area, cane or stalk yield and total production with rates of approximately 40, 10 and 50%, respectively (Figure 1.1). Currently, the world sugarcane production is quite stable, which occupies a total harvested area of around 26.8 Mha (million hectares) and produces a total production of about 1,890 Mt. (million tons) (FAOSTAT, 2016). The world sugar demand is predicted to increase by 16.2% to over 53 million metric tons in the next ten years (Taylor, 2017). Moreover, world ethanol production is projected to increase annually by 0.7%, and the contribution of sugarcane becomes more and more important when sugarcane used for biofuel will increase up to 20%, meanwhile contribution of other biofuel sources (maize and vegetable oil) will decrease to below 15 and 12%, respectively (Goswami, 2018). Hence, to meet above demands, the global sugarcane production is projected to increase to exceed 2,200 Mt. on the total harvested area of 30 Mha (The Statistics Portal, 2018; OECD-FAO, 2016).

Sugarcane is indigenous to South and Southeast Asia, then it has been distributed around the world between the latitude 36.7^o N (southern Spain) and 31.0^o S (South Africa) of the equator from tropical to subtropical zones. Most of the sugar (>75%) and sugarcane (>95%) producers come from developing countries (FAOSTAT, 2016; OECD-FAO, 2016). Brazil (768.7 Mt.), India (348.4 Mt.), China (122.7 Mt.), Thailand (87.5 Mt.), Pakistan (65.5 Mt.), Mexico (56.4 Mt.) Colombia (37.0 Mt.), Australia (34.4 Mt.), Guatemala (33.5 Mt.), United State (29.9 Mt.), Indonesia (27.2 Mt.), Philippines (22.4 Mt.), Argentina (22.0 Mt.), Cuba (18.9 Mt.) and Vietnam (16.3 Mt.) are in the top 15th world sugarcane producing countries which accounts for approximately 90% of the global sugarcane production.

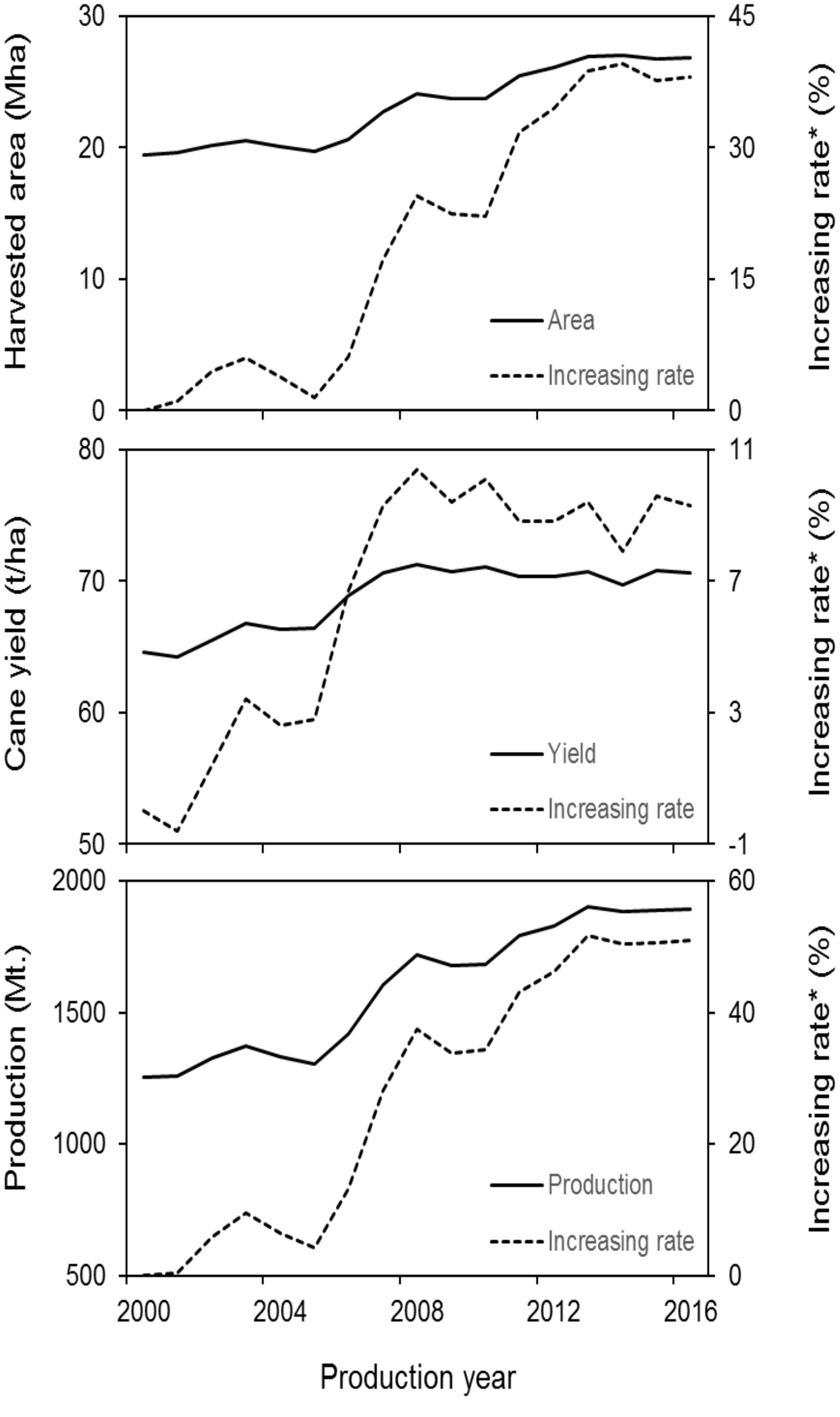

Figure 1.1. Sugarcane production in the world during the first years of the 21st century

Source: FAOSTAT, 2016. ** Increasing rate compared to production data in the year 2000*

1.2. Drought stress and its effects on sugarcane production

Sugarcane produces enormous biomass with an average of 40 tons ha^{-1} year^{-1} (Waclawovsky et al., 2010). Moreover, the green tissues contain 60 - 80% water, in sugarcane's life cycle it requires a huge water amount with at least 1,500 - 2,000 mm per year of rainfall to create an optimum production (FAO, http://www.fao.org). Most sugarcane areas have been grown in tropical and subtropical zones under rain-fed conditions where low rainfall and prolonged dry spells during crop growth periods may be the main constraint to sugarcane production. Thus, irrigation is often required to maintain high yield and production. However, most sugarcane productions are produced in developing countries with low inputs where almost water resource depends on rainfall, irrigation is always not available. For instance, only about 1% of the total sugarcane areas in Brazil is currently irrigated, and about 70% of cultivated lands locate in high water deficit regions (Laclau and Laclau, 2009). More than 80% of sugarcane production in China are grown in upland areas without irrigation (Li and Yang, 2015). In India, more than 60 - 70% of the country areas are vulnerable to drought stress (Jadhav, 2018), which bring down the yields by 30 - 50% (Solomon, 2016). Similarly, almost cane-growing areas in Thailand are under rain-fed conditions with only 10% are grown in irrigated conditions (Manivong and Bourgois, 2017). Nevertheless, about 60% of the sugarcane produced in Australia depend on irrigation to some extent (Carr and Knox, 2011), but this practice always results in high production cost (Zingaretti et al., 2012). Therefore, drought stress has become the main limiting factor in sugarcane production.

1.3. Effect of drought stress on sugarcane growth

Similar to other shallow root system crops in Poaceae family, sugarcane root system closes to the soil surface with approximately 50% of root biomass occurring in the top 20 cm and 85% in the top 60 cm and then decreasing exponentially with depth (Smith et al., 2005). Low soil humidity, which firstly occurs at the topsoil layer when plant subjects to drought stress, affects water absorption, root water status (Zingaretti et al., 2012) and root growth which is recorded in the reductions of root length, root surface area, root volume and root biomass (Barbosa et al., 2015; Jangpromma et al., 2012; Wagih et al., 2003). Because the stomatal and root hydraulic conductance are correlated in sugarcane, the changing in root water status may affect leaf water potential and assimilation (Smith et al., 2005). Drought stress reduces assimilation rate or photosynthetic activities (Jangpromma et al. 2010; Graça et al., 2010;

Barbosa et al., 2015) leading to lacking energy and essential materials to support elongation cells and other growth processes, particularly in terms of a decrease in cell elongation and cell volume (Nonami, 1998; Larcher, 2003). As the result, it causes the reductions in leaves and stalks growth which directly relate to dry matter of above parts as well as whole plant biomass and cane yield (Ramesh, 2000; Robertson et al., 1999; Zhang et al., 2015; Zhao and Li, 2015), and finally in sucrose accumulation (Inman-Bamber, 2004).

1.4. Drought stress at different growth stages

In general, the sugarcane growing season can be divided into four growth stages: i) initial stage, from sowing to 10% ground cover; ii) developmental stage, from 10% to 70% ground cover; iii) mid-season stage including flowering and yield formation; and iv) late-season stage (Ellis and Lankford, 1990; Ethan et al., 2016). Meanwhile, Gascho and Shih (1983) divided sugarcane growing season into four developmental stages: i) germination stage, the development of buds and roots; ii) tillering stage, issuance of secondary and tertiary tillers; iii) grand growth stage, tillers growth and development with height gain and basal sugar accumulation; and iv) maturity stage, accumulation of photo-assimilates and fast sugar synthesis, lasting until the harvesting period (Zingaretti et al., 2012). In this study, I considered early growth stage or formation stage including the tillering phase and early elongating or early grand growth phase.

The effects of drought stress on growth and productivity of sugarcane are varying according to crop growth stages, duration, and level of water deficit. While water stress at early and mid-season stages reduces growth, cane and sugar yield, late-season drought stress at moderate levels in last two months does not affect cane yield, and somewhat favorite to sucrose accumulation and improving sugar yield (Abdel-Wahab, 2005; Ethan et al., 2016). However, prolonged drought stress at late-season may also reduce cane yield. Similarly, while having large impacts on leaf area, tillering and dry matter accumulation, drought stress has a little effect on final yield because of recovery after a short drought duration at tillering phase (Robertson et al., 1999). In sugarcane, tillering and grand growth are critical phases of drought sensitivity due to the high water requirement for sustainable growth (Ramesh, 2000; Zingaretti et al., 2012), and because 70 to 80% of the total cane yield are produced during these phases (Singh and Rao, 1987; Zhao et al., 2010). Depending on levels of drought stress, the reductions in dry matter content are different by over 60, 50 and 25% under severe level, and by over 45, 35 and 15%

under moderate drought at the end of the formation, grand growth and maturity phases, respectively (Ramesh, 2000).

1.5. Different responses of sugarcane varieties to drought stress

Although water deficit can lead to losing sugarcane productivity by up to 60% (Robertson et al., 1999), the reduction rates are different depending on the variation in responses of sugarcane varieties. Basnayake et al. (2012) reported that water stress reduced cane yield and total dry matter of eighty-nine genetically diverse sugarcane clones by 17 - 52% and 20 - 56%, respectively. In general, under stress conditions, drought tolerant genotypes maintain higher productivity, stalk number, stalk height and diameter, cane and sugar yield than the susceptible ones do (Begum and Islam, 2012; Hemaprabha et al., 2013; Ribeiro et al., 2013, Silva et al., 2008). Drought tolerant genotypes also perform better plant growth and photochemical capacity. For instance, sugarcane genotypes with the tendency to develop deeper and larger root system also have higher drought tolerant ability (Endres et al., 2010; Ferreira et al., 2017; Jangpromma et al., 2012; Smith et al., 2005; Wagih et al., 2003). These genotypes also have better leaf growth and physiological parameters such as photosynthetic rate, quantum efficiency photosystem II, stomatal conductance, transpiration rate, chlorophyll content, leaf area, and relative water content (Basnayake et al., 2015; Begum et al., 2012; Graça et al., 2010; Mederios et al., 2013; Silva et al., 2013; Zhao et al., 2013). Moreover, better drought tolerant genotypes could lighten the damaging effects of drought on gas exchange, and maintain high dry matter production as well as have better recovery ability from mild stress (Mederios et al., 2013). Drought tolerant cultivar also exhibits one additional active superoxide dismutase isoenzyme in comparison with commercial cultivar. The total glutathione reductase activity also increases substantially in drought tolerant cultivar under conditions of severe water stress (Boaretto et al., 2014). In the opposite way, drought sensitive genotypes have a large chlorophyll degradation, low photosynthetic abilities; and higher reduction rates of biomass and leaf area (Cha-um and Kirdmanee, 2008). The highest lipid peroxidation, hydrogen peroxide, and proline contents during the progression of the drought stress conditions are exhibited, whereas superoxide dismutase isoenzyme is absent at the end of drought stress period in sensitive cultivars (Cia et al., 2012). The number of expressed genes in the sensitive cultivar is higher, and increasing with the severity of water deficit in comparison with those in the tolerant cultivar. Moreover, 45% of the genes expressed in sensitive one are down-regulated, while 94% of genes expressed in drought tolerant one is up-regulated under water stress conditions (Rodrigues et al., 2009).

1.6. Role of nitrogen and nitrogen use efficiency of sugarcane

Nitrogen (N) plays an important role in sugarcane crops. It involves in many critical processes such as plant growth, expansion of green leaves, and tiller or sucker production, especially in the formation of plant protein, which is essential for photosynthesis such as PEPcase or Rubisco (Schroeder et al., 2014). Deficiency of N, therefore, will be the reason for reductions in plant growth, cane and sugar yield. The reduction of photosynthetic capacity by N deficiency, namely in stomatal conductance, Rubisco, and PEPcase activity and partitioning of carboxylase activity to Rubisco relative to PEPcase leading to declining in quantum yield were reported in sugarcane by Ranjith (1994), and Meinzer and Zhu (1998). Similarly, Kumara and Bandara (2001) reported that lack of N application caused the reductions in leaf area index, photosynthetic rate, leaf N content, leaf chlorophyll content and biomass by approximately 28, 18, 28, 10 and 13%, respectively. In normal, applying N results in high plant height, stalk population, stalk length, cane and sugar yield, and improving bud sprouting (Rosa et al., 2015; Sime, 2013; Wiedenfeld, 1995; Wiedenfeld and Enciso, 2008). However, in many cases, higher N application does not have any significant effect on shoot biomass and the root density (Otto et al., 2014), dry matter yield (Ishikawa et al., 2009), cane yield, sugar yield and sugar quality (Koochekzadeh et al., 2009; Madhuri et al., 2011; Muchovej and Newman, 2004). Excess N application even leads to prolonging vegetative growth and reducing sugar yield and quality (Bell et al., 2014, Hemalatha, 2015). Therefore, the optimum dose or better nitrogen use efficiency (NUE) is more important than increasing N application. In fact, Acreche (2017) demonstrated the positive association between NUE traits with sugar yield and cane yield. Similarly, Calif and Edgecombe (2015) showed remarkable improvements in plant growth and biomass production in sugarcane lines incorporating NUE traits.

1.7. Variation in nitrogen use efficiency of sugarcane varieties

The response and NUE of sugarcane varieties are different among N application levels. Obviously, at the optimum N dose, sugarcane plant often has the highest NUE as well as cane and sugar yield (Koochekzadeh et al., 2009; Saleem et al., 2012). However, because of N deficiency, higher NUE at low N level somewhat is not synonymous with higher cane and sugar yield in comparison with lower NUE at higher N level (retrieving from Saleem et al. (2012)'s data). Similarly, Ranjith and Meizer (1997) found an increase of photosynthetic NUE under N stress conditions, but in the same N level, PNUE of N stress tolerant variety is always higher than that of sensitive one. Evidence for difference of NUE in sugarcane varieties are reported by Hajari et al. (2015), Robinson et al. (2009) and Schumann et al. (1998) under *in vitro*, glasshouse, and experimental field conditions, respectively. However, the response, as well as NUE traits of sugarcane varieties, are quite different from N sources. Several varieties respond well to N additions and produce less than 50% of the biomass under zero N addition compared with added N fertilizer. Meanwhile, several ones are less responsive to N supply when grown without added N fertilizer producing up to 70% of the biomass at high N supply (Robinson et al., 2009). Similarly, some varieties had the same NUE response under *in vitro* conditions, but another one behaves differently to various N levels and N forms (Hajari et al., 2015).

1.8. Hypothesis and objectives of the study

Tillering and grand growth phases are critical stages of drought sensitivity due to the high water requirement and contribution to sustainable growth in sugarcane. Although the grand growth stage is the longest stage with highest water requirement, by crop season management it is often fixed closely during the rainy season, hence, the effect of drought stress during this stage is diminished. Therefore, water stress at tillering and early grand growth (early growth stage) become more frequent. Unfortunately, N is often applied at this stage and finishing before plant elongating to promote sugarcane growth. Drought stress during this stage, hence, reduces uptake of N (Silva et al., 2017) and NUE. Because NUE traits positively correlated to sugar and cane yield (Acreche, 2017), the reduction in NUE will result in yield loss and low sugar quality.

The hypothesis in this study is that improvement of NUE under drought stress condition could be a remedy to rescue sugarcane production. In fact, Calif and Edgecombe (2015) showed remarkable improvements in plant growth and biomass production in sugarcane lines incorporating NUE traits. In maize, drought tolerant cultivars produced consistently higher yields because these cultivars have either high N uptake or N utilization efficiency (Kamara et al., 2014). In sweet sorghum, improved water and NUEs under water stress may both contribute to the high degree of physiological acclimation to drought (Wang et al., 2014). In sugarcane, Ranjith and Meizer (1997) found that NUE was significantly higher in drought resistant genotype than in the susceptible genotype. Souza and Vitorello (2014) also found that a sugarcane cultivar with higher PNUE displayed less metabolic inhibition of photosynthesis during drought. Therefore, higher NUE traits should be a strategy to confront with the compromise from a shortage of water source. However, still, no information has been reported on the relationship between NUE and drought tolerant ability in sugarcane.

The present study focuses on the photosynthetic and agronomical responses of sugarcane to drought stress conditions under different N applications, with various sugarcane varieties to determine the relationship between NUE with drought tolerant ability to get a better understanding of the mechanism of drought stress tolerance in sugarcane.

Three experiments were carried out:

- To demonstrate sugarcane plants with better NUE traits could have better tolerant ability to drought stress at early growth stage with different N application levels (Chapter 2);
- To demonstrate better NUE traits could be the strategy to sugarcane varieties confront to drought stress at early growth stage (Chapter 3);
- To suggest high NUE sugarcane variety by screening commercial and selected sugarcane varieties under the field trail throughout drought stress at early growth stage (Chapter 5).

One more experiment to investigate irrigation time to keep acceptable sugarcane growth based on the daily change in soil moisture content and photosynthesis of sugarcane when plant subjected to water deficit and after re-watering was also conducted (Chapter 4).

CHAPTER II

NITROGEN USE EFFICIENCY OF SUGARCANE UNDER DROUGHT STRESS AT EARLY GROWTH STAGE

2.1. Photosynthetic response and nitrogen use efficiency of sugarcane under drought stress conditions with different nitrogen application levels

2.2.1. Introduction

Drought stress is one of the main factors constraining sugarcane production in over the world. Water deficit which restricts growth and photosynthetic activity (Jangpromma et al., 2010; Graça et al., 2010; Barbosa et al., 2015) is responsible for the reduction in biomass and cane yield (Ramesh, 2000; Zhao and Li, 2015). However, the effects of drought stress are varying according to crop growth stages. Drought stress at early and mid-season stages reduced growth, cane, and sugar yield, whereas cane and sugar yield produced when water stress imposed at the late-season stage was not significantly from yields obtained at full irrigation (Ethan et al., 2016). Tillering and grand growth phases were critical stages of drought sensitivity due to the high water requirement for sustainable growth in sugarcane (Zingaretti et al., 2012). Although the grand growth stage is the longest stage with highest water requirement, by crop season management it is often fixed closely during the rainy season, hence, the effect of drought stress in this stage is diminished. Therefore, water stress at tillering and early grand growth (early growth stage) become more frequent.

N plays an important role in sugarcane crops. It involves in many critical processes such as plant growth, expansion of green leaves, and tiller or sucker production, especially in the formation of plant proteins which is essential for photosynthesis such as PEPCase or Rubisco. The growth and yield of sugarcane are fallen by the deficiency of N, but excess N can lead to prolonging vegetative growth and reducing sugar yield and quality (Bell et al., 2014). Higher NUE can support better growth and higher crop yield. For instance, increase N uptake and NUE in rice contributed to the increase in grain yield (Zhu et al., 2016). In sugarcane, the positive association was found among NUE traits with sugar yield and cane yield (Acreche, 2017). Similarly, Calif and Edgecombe (2015) showed remarkable improvements in plant growth and biomass production in sugarcane lines incorporating NUE traits.

Drought stress restricted nutrient uptake may cause a deficiency of nutrient, or rather, N (Dinh et al., 2014). Unfortunately, N is often applied at early stage and finished before plant elongating. Therefore, uptake of N and NUE may be fallen due to water stress during early growth stage. In maize, drought tolerant cultivars produced consistently higher yields because these cultivars had either high N uptake or N utilization efficiency (Kamara et al., 2014). In sweet sorghum, improved water use efficiency and NUEs under water stress may both contribute to the high degree of physiological acclimation to drought (Wang et al., 2014). Therefore, higher NUE should be a strategy to confront with the compromise from a shortage of water source. However, still, no information has been reported on NUE under drought period in sugarcane.

The present study focuses on the photosynthetic and agronomical responses of sugarcane to drought stress conditions under different N applications, and the relationship between NUE under drought conditions with drought tolerant index to get a better understanding of the mechanism of drought stress tolerance in sugarcane.

2.1.2. 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 126 m) from May to September 2016. Two months old seedlings of commercial cultivar NiF8 were transplanted into Wagner pots (1/2,000a) filled with 10 kg substrate of red soil: sea sand: peat moss (1:1:1, v v^{-1}) at 12% of gravimetric soil moisture content. The substrate properties were analyzed before transplanting with pH (7.1), electronic conductivity (153.1 mS m^{-1}), total N (0.07%), P (0.1 ppm) and K (12.2 ppm).

A split-plot design was used with three replications. Two soil water regimes including well-watered (WW) at field capacity and drought stress (DS) at 1/3 of available water for 60 days were assigned in main plots. In subplots, four N levels including 0, 4.4, 8.8 and 13.2 g ammonium sulfate pot^{-1} (equivalent to 0, 90, 180 and 270 kg N ha^{-1} in field conditions, respectively) were designed.

Experimental pots were arranged in distance 40 x 90 cm of each pot and row. During the experiment period, all tillers were removed immediately after emergence. At 7 days after transplanting (DAT), superphosphate at rate 5.2 g pot^{-1} was applied. Potassium chloride (2.1 g pot^{-1}) and ammonium sulfate were fertilized at the same ratio of 1: 1.5: 1: 1.5 at 7, 30, 60 and 90DAT, respectively.

As soon as transplanting, irrigation with full available water was practiced until 60DAT. After that at the moisture stress plot, water was applied by just 50% of water loss until soil moisture content reaching to 1/3 available water (it took 10 days), then by full water loss of this treatment for another 50 days. Pot weight of each treatment was determined every day using a gravimetric balance to calculate the amount of water loss. Soil moisture content (37.19% of field capacity and 28.36% of 1/3 available water) was monitored by Hydra probe soil sensors (Steven Water Monitoring Systems, Inc., Portland, OR) at 10 cm of depth with corresponding values for field capacity and 1/3 available water were 0.40 and 0.29 m^3 m^{-3}, respectively (Figure 2.1).

Data collections

An open gas exchange system, in detail described by Kawamitsu et al (2002), were used to determine photosynthetic light response curve at PFD (50 to 1500 µmol m^{-2} s^{-1}, at 400 µmol mol^{-1} CO_2) and A/C_i curve at various CO_2 concentration (30 to 1000 µmol mol^{-1} CO_2, at 1500 µmol m^{-2} s^{-1} of PFD). At one week before the start and completion of the drought stress period, one sample plant of each treatment was taken randomly. The first fully expanded leaf from the top of each sampled plant was set in a flexible chamber (26 x 30 x 9cm). Leaf temperature was controlled at 30 ± 1°C.

From 5DAT, the growth parameters including plant height and total leaves number of each treatment were collected at each one-week interval. At one day before the start and complete of drought treatment, the first fully expanded leaves of sample plants of each treatment in all replications were taken to determine potential photosynthetic rate (A_{max}), stomatal conductance (g_s) and transpiration rate by LI-6400 portable photosynthesis system (LI-COR, Lincoln, Nebraska, USA) equipped with a 2x3 cm^2 LED chamber between 0900 to 1500 at a PFD of 2000 µmol m^{-2} s^{-1}, leaf temperature of 31±2°C, CO_2 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).

The first leaf of each sampled plant was cut to determine leaf area by LI-3100 leaf area meter (LI-COR, Lincoln, Nebraska, USA), then oven-dried at 80°C for 48hr to determine dry weight. After grinding by TI-100 vibrating sample mill (CMT, Tokyo, Japan), 25 mg of dry leaf sample was taken to determine specific leaf N content (NL) by an N/C analyzer (NC-90A, Shimadzu, Japan).

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

PNUE = Photosynthetic rate/specific leaf N content;

PWUE = photosynthetic rate/transpiration rate.

At 120DAT when drought stress treatment was completed, the sample plant was cut by separating leaves, stem, and root. The stem (after squeezing) and leaves (after scanning by LI-3100) were dried at 80°C for 48hr to determine to shoot dry weight. The root was cleaned by tap water, then drying at 80°C for 48hr to determine root mass. Total biomass was calculated by the sum of the shoot and root dry weight. Then, biomass nitrogen use efficiency (BNUE) was calculated as follows:

BNUE = total biomass production/ N applied amount

Drought tolerant index (DTI) was determined as follows:

DTI = biomass under stress condition/ biomass under well-watered 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 photosynthetic traits and biomass were calculated to assess the relationships.

2.1.3. Results and discussion

Meteorological conditions and soil moisture content

Meteorological conditions and soil moisture content (soil moisture volume fraction) in the experimental site were shown in Figure 2.1. During the experimental time, the air temperature and air humidity in glass house ranging from 20.0 to 42.0°C and from 86.6 to 98.3%, which were higher 2.04 ± 0.7°C and 12 ± 2.5% than those at outside, respectively. Average daily solar radiation ranged from 10 to 180 W m^{-2}, which was as 0.53 times as this parameter at outside condition (Figure 2.1a). The soil moisture content of control treatment fluctuated around 0.38 m^{3} m^{-3}. Meanwhile, in stress treatments, soil moisture content stabled around 0.38 m^{3} m^{-3} until 60DAT (before stress treatment), then reduced rapidly and changed around 0.29 m^{3} m^{-3} from 70 to 120DAT (Figure 2.1b). During late period of drought stress, soil moisture was more fluctuated in both control and water stress treatments because of higher air temperature but lower air humidity, and since higher water requirement to compensate for water loss from larger growth plant.

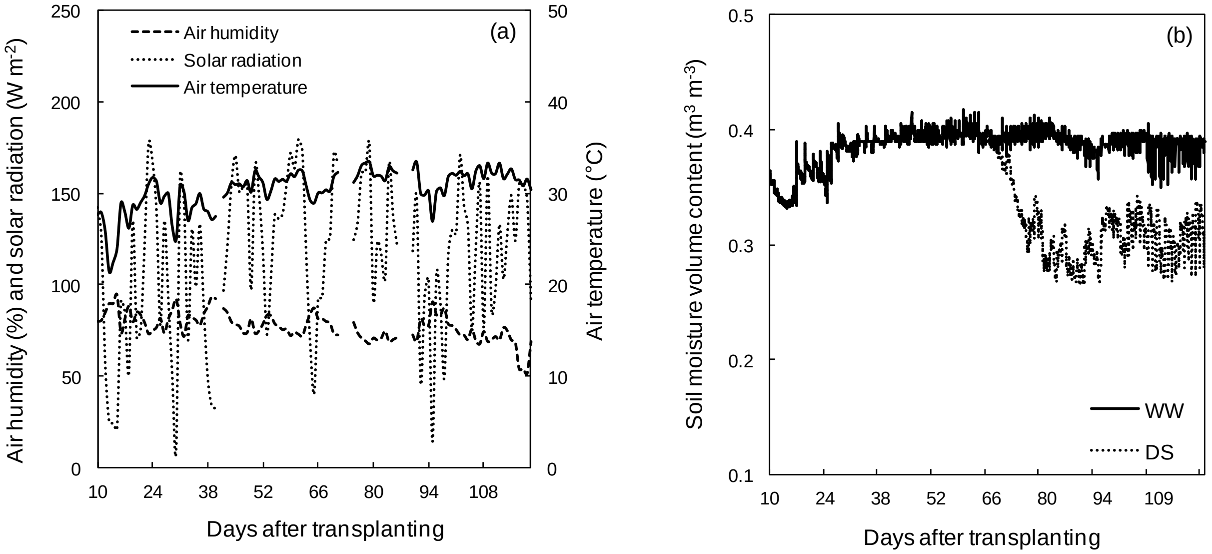

Figure 2.1. Weather conditions (a) and soil moisture content (b) at well-watered (WW) and drought stress (DS) treatments during the experimental period.

Photosynthetic responses of sugarcane to light intensity and CO_2 concentration

An initial slope of photosynthetic light response curve showed that photosynthesis is limited by the concentration of chlorophyll and the efficiency of light dependent reactions, but in higher levels, it is limited by the efficiency of Rubisco and the availability of CO_2. As can be seen from Figure 2.2, there were upward trends in photosynthetic rate (*A*) when light intensity increased at all N levels and both water regimes. In fact, at fertilized treatment, *A* rose rapidly, then slowly when PFD reached approximately 1000 µmol m^{-2} s^{-1}. The same trends were found in previous studies in another C_4 plant *Amaranthus retroflexus* (Sage and Pearcy, 1987), and sugarcane under normal and mild stress conditions (Allison et al., 1997; Zhao et al., 2013; Sage et al., 2014).

An A/C_i curve shows the photosynthetic response of leaf tissue to varying intercellular CO_2 concentrations. In C_3 plant, the shape of photosynthesis is limited by Rubisco capacity on the initial slope and by RuBP regeneration capacity at saturated CO_2 concentration. Meanwhile, in C_4 plant it is limited by PEPCase on the initial slope, but very complex at CO_2 saturation with effects by Rubisco, RuBP generation or PEP regeneration capacity (Sage et al., 2014).

A/C_i curve shown in Figure 2.2 illustrated that A increased when internal CO_2 concentration (C_i) increased at all N levels and both water regimes. In fact, A increased rapidly then became stable when C_i reached approximately 200 µmol mol^{-1}. However, 200 µmol mol^{-1} seemed to be the saturated point of C_i just in case of the photosynthetic rate of sugarcane grown under well-watered conditions and fertilized drought stress conditions. This finding is in line with other previous studies in sugarcane (Du et al., 1996; Sage et al., 2014), sorghum (Zhao et al., 2005) or another C_4 plant, *Cenchrus ciliaris* and *Panicum coloratum* (Ghannoum, 2009), *Tidestromia oblongifolia* (Berry and Downton, 1982).

In 0N treatment, the photosynthetic response was a little different to fertilized treatments. Under normal condition, A increased slowly then became stable from 1000 µmol m^{-2} s^{-1} of PFD (Figure 2.2c). This photosynthetic response was also shown by Sage and Pearcy (1987) with *Amaranthus retroflexus* at lower levels of N, and Zhao et al. (2013) with sugarcane under severe drought stress. Under drought condition, A even decreased after reaching a peak at around 600 µmol m^{-2} s^{-1} of PFD (Figure 2.2e), whereas it did not cease after C_i reached 200 µmol mol^{-1} (Figure 2.2f). It seemed to be that photosynthetic activity of sugarcane, a C_4 plant to be lessening with a response as close as the C_3 plant or a C_3-C_4 intermediate species (as shown by Monson, 1999) when plants confront the severe stress from N and/or moisture deficiency. It may lead to lower PNUE of 0N treatment compared to fertilized treatments because of PNUE of C_4 plant is generally higher than the C_3 plant (Sage and Pearcy, 1987).

Effect of drought stress and nitrogen application on photosynthesis of sugarcane

A_{max} reduced from 60DAT to 120DAT at all N applied treatments (Table 2.1a, b). Consuming from earlier studies, McCormick et al. (2006) reported that the photosynthetic rate of sugarcane had been related to plant age which young plants assimilated higher rates than older ones did. Inman-Bamber et al. (2011) also found that the photosynthetic rate of the whole plant and of single leaves decreased up to 60% in some cases. In this study, lower A_{max} at later period might because of lower NL in comparison with the earlier period (Table 2.1a, b). Linking to specific leaf N content, Allison et al. (1997) also agreed that the photosynthetic rate increased linearly to NL when NL ranged from less than 1.0 to 1.7 g m^{-2}.

Photosynthetic traits including initial slope of A/C_i curve (IS), A_{max}, g_s, SPAD and NL increased with the increase of N application levels (Table 2.1a, b). In fact, A_{max} of 180N and 270N treatments, in normal, were significantly higher than that of 90N, but not significant when plant subjected to drought conditions. In contrast, the significant differences for g_s, SPAD and NL were just found at 60DAT and in NL under drought conditions at 120DAT. Meanwhile, 0N

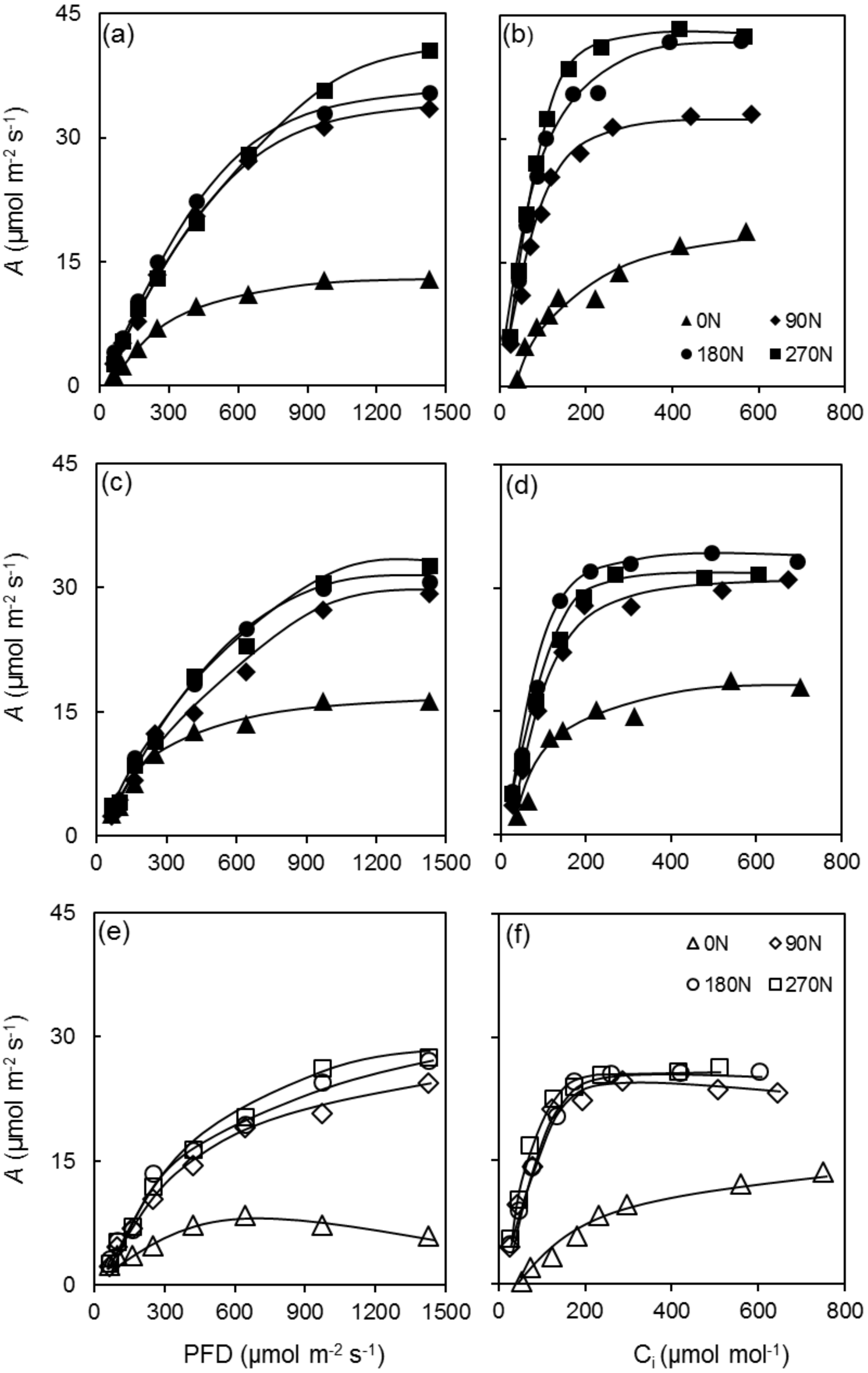

Figure 2.2. Light and A/C_i response curves at 60DAT (a, b); and at 120DAT under well-watered (closed shapes) (c, d) and drought stress (opened shapes) (e, f) conditions with different nitrogen application levels.

had lower values for all traits comparing to fertilized treatments under both conditions. Higher NL was a result of L was a result of higher nitrate reductase activity by increasing N supply (Abayomi et al., 1988; Abayomi, 2001; Wang et al., 2014). Due to the positive correlations among NL with both Rubisco and PEPCase activities (Meinzer and Zhu, 1998), higher IS were found in higher N application treatments which enhanced a higher A_{max} than lower N applied treatments. This was demonstrated by the positive correlations between NL and IS (Figure 2.3a, c), between IS and A_{max} (Figure 2.3b, d). Furthermore, the correlation between NL and A_{max}, gs and SPAD were positive significant (Figure 2.4). These results suggested that higher N application supported higher A_{max}, g_s and chlorophyll content (or SPAD) because of higher N concentration accumulated into the leaf.

In the earlier period (at 60DAT), PNUE reduced along with the increasing dose of N application (Table 2.1a). Nevertheless, at 120DAT, PNUE increased significantly from 0N to 90N, then slightly climbed under the well-water condition, but decreased under drought stress condition (Table 2.1b). At both periods, PWUE of fertilized treatments were not different and higher than those of 0N treatment. Under field condition at 300 days after planting, Oliveira et al. (2016) also found that sugarcane added N in irrigation system induced an increase of PWUE by 14.75% in comparison with that did not receive N. A_{max} increased with a polynomial regression rather than linear type with increase of NL (Figure 2.4; Meinzer and Zhu, 1998). As can be seen from Figure 2.4, A_{max} reduced when NL reached approximately 1.71 g m^{-2} at 60 DAT and 1.0 g m^{-2} at 120DAT, respectively. This is along with Allison et al. (1997) that *A* and NL had linear regression just in case of NL less than 1.7 g m^{-2}. Furthermore, very high NL in high N fertilized treatment as 5 times as 0N treatment were found at 60DAT, whereas these were just 2 times at 120DAT might be the reason for the decrease of PNUE when N was applied at the earlier stage (Figure 2.5a). Wang et al. (2014) found the same point with a significant reduction of PNUE in a nonlinear relationship with NL of sweet sorghum seedlings.

Excepting for PWUE and NL, drought reduced IS, A_{max}, g_s, PNUE and SPAD (Table 2.1b). Jangpromma et al. (2012) showed a non-significant effect of water stress on water use efficiency of 10 sugarcane cultivars in Thailand. At 300 day after planting, different water replacements did not significantly alter PWUE (Oliveira et al., 2016). Reduction of nitrate reductase activity under water stress conditions (Abayomi et al., 1988) might be responsible for the decline of NL (Wang et al., 2014). However, the depressive effect of moisture stress was found in three of six cultivars, whereas another cultivar NL did not differ or even higher at rain-fed treatment compared to irrigated treatment (Ludlow et al., 1991). In fact, Abayomi et al. (1998) also found the reduction in nitrate reductase activity by the effect of drought when increasing N application in Co 957 cultivar, whilst nitrate reductase activity still increased with N supply in Co997. In this study, after drought stress period, the redundancy of N from former in company with smaller leaf area (Table 2.1b), might be the reason for later higher NL in 180N and 270N treatments. In the opposite way, the deficiency of N in lower N application treatments might cause decreases of NL. Many previous studies discovered the same effects of drought stress on photosynthetic rate, g_s, and chlorophyll content or SPAD value of sugarcane (Du et al., 1996; Koonjah et al., 2006; Silva et al., 2007; Silva et al., 2011; Jangpromma et al. 2010; Graça et al., 2010; Barbosa et al., 2015). In the C_4 plant, the initial slope of photosynthetic response shows the activity of PEPCase and effect of chlorophyll content in leaves, whereas, at higher levels of light and A/C_i curve, the photosynthetic rate may affect by RuBP regeneration. Rubisco and PEPCase activity, main limiting factors for photosynthesis, decreased when sugarcane subjected to drought stress (Du et al., 1996), as a result, it caused reductions in IS and A_{max}. Previous studies used the slope value of line regression in the relationship between A_{max} and NL as PNUE (Sage and Pearcy, 1987; Sage et al., 2014). By this way, drought stress also caused reductions in PNUE of sugarcane because of the slopes values were 46.0 and 24.9 (Figure 2.4) under well-watered and stress conditions, respectively. This result was consistent with lower PNUE values of drought stress treatment in Table 2.1b.

Table 2.1a. The initial slope of A/C_i curve (IS), potential photosynthetic rate (A_{max}), stomatal conductance (g_s), photosynthetic nitrogen use efficiency (PNUE), photosynthetic water use efficiency (PWUE), SPAD and specific leaf nitrogen content (NL) across water regimes with different nitrogen application levels at 60DAT

N levels	IS	A_{max} µmol m^{-2} s^{-1}	g_s mol m^{-2} s^{-1}	PNUE µmol s^{-1} g^{-1}	PWUE µmol mol^{-1}	SPAD	NL g m^{-2}
0N	0.068	26.6^{c}	0.20^{b}	39.9^{a}	5.7^{b}	30.5^{c}	0.66^{c}
90N	0.145	35.5^{b}	0.24^{b}	36.9^{ab}	6.5^{a}	36.0^{c}	0.97^{c}
180N	0.201	41.5^{ab}	0.29^{b}	29.5^{bc}	6.5^{a}	44.2^{b}	1.45^{b}
270N	0.238	45.0^{a}	0.32^{a}	24.4^{c}	6.5^{a}	50.4^{a}	1.86^{a}
Source of variance							
Water regimes (W)	-	ns	ns	ns	ns	ns	ns
N levels (N)	-	**	*	**	*	**	**
W*N	-	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 among N applied levels at $P < 0.05$ by Turkey.

Table 2.1b. Initial slope of A/C_i curve (IS), potential photosynthetic rate (A_{max}), stomatal conductance (g_s), photosynthetic nitrogen use efficiency (PNUE), photosynthetic water use efficiency (PWUE), SPAD and specific leaf nitrogen content (NL) under well-watered and drought stress conditions with different nitrogen application levels at 120DAT

Water regimes	N levels	IS	A_{max} µmol m^{-2} s^{-1}	g_s mol m^{-2} s^{-1}	PNUE µmol s^{-1} g^{-1}	PWUE µmol mol^{-1}	SPAD	NL g m^{-2}
Well-watered	0N	0.072	16.0^{c}	0.13^{b}	28.6^{b}	5.2^{b}	27.6^{b}	0.56^{b}
	90N	0.143	27.9^{b}	0.17^{a}	36.3^{a}	6.4^{a}	36.4^{a}	0.77^{a}
	180N	0.151	30.7^{a}	0.21^{a}	38.5^{a}	6.5^{a}	37.3^{a}	0.80^{a}
	270N	0.144	31.5^{a}	0.19^{a}	36.5^{a}	7.1^{a}	39.3^{a}	0.86^{a}
	Average	0.128	26.5^{A}	0.17^{A}	35.0^{A}	6.3	35.2^{A}	0.73
Drought stress	0N	0.043	8.9^{b}	0.08^{b}	16.8^{b}	4.5^{b}	17.2^{c}	0.54^{b}
	90N	0.105	19.5^{a}	0.11^{ab}	29.1^{a}	7.7^{a}	31.4^{b}	0.67^{b}
	180N	0.131	23.3^{a}	0.13^{a}	26.1^{a}	6.9^{a}	35.5^{a}	0.90^{a}
	270N	0.123	22.8^{a}	0.12^{a}	22.2^{ab}	7.8^{a}	34.0^{ab}	1.03^{a}
	Average	0.101	18.7^{B}	0.11^{B}	23.6^{B}	6.7	29.5^{B}	0.78
Source of variance								
Water regimes (W)		-	*	**	**	ns	*	ns
N levels (N)		-	**	*	**	**	**	**
W*N		-	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 among N applied levels at $P < 0.05$. Different capital letters in the same column show significance between water conditions at $P < 0.05$ by Turkey.

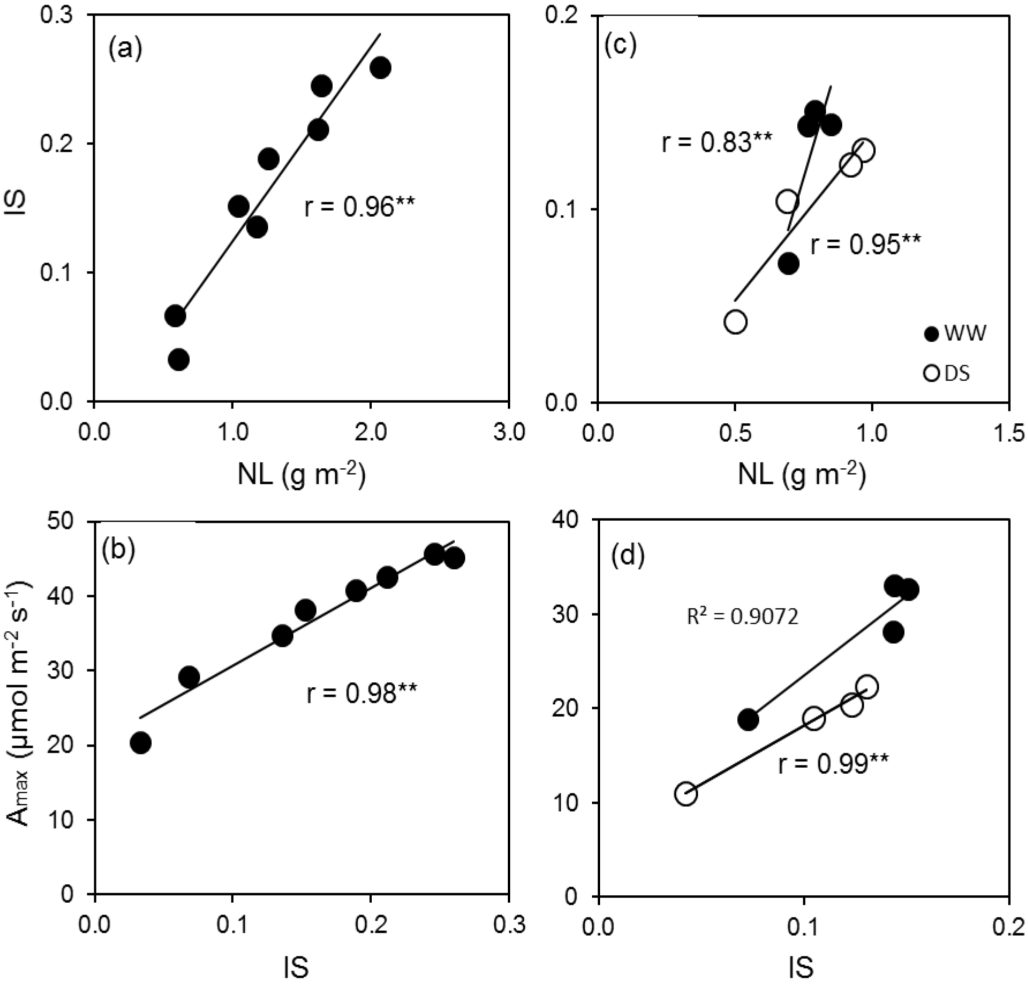

Figure 2.3. The correlation between specific leaf nitrogen content (NL) and initial slope of A/C$_i$ curve (IS) (a); and between IS and potential photosynthetic rate (A$_{max}$) (b) at 60DAT; and at 120DAT (c, d, respectively) under well-watered (closed shape) and drought stress (opened shape) conditions.

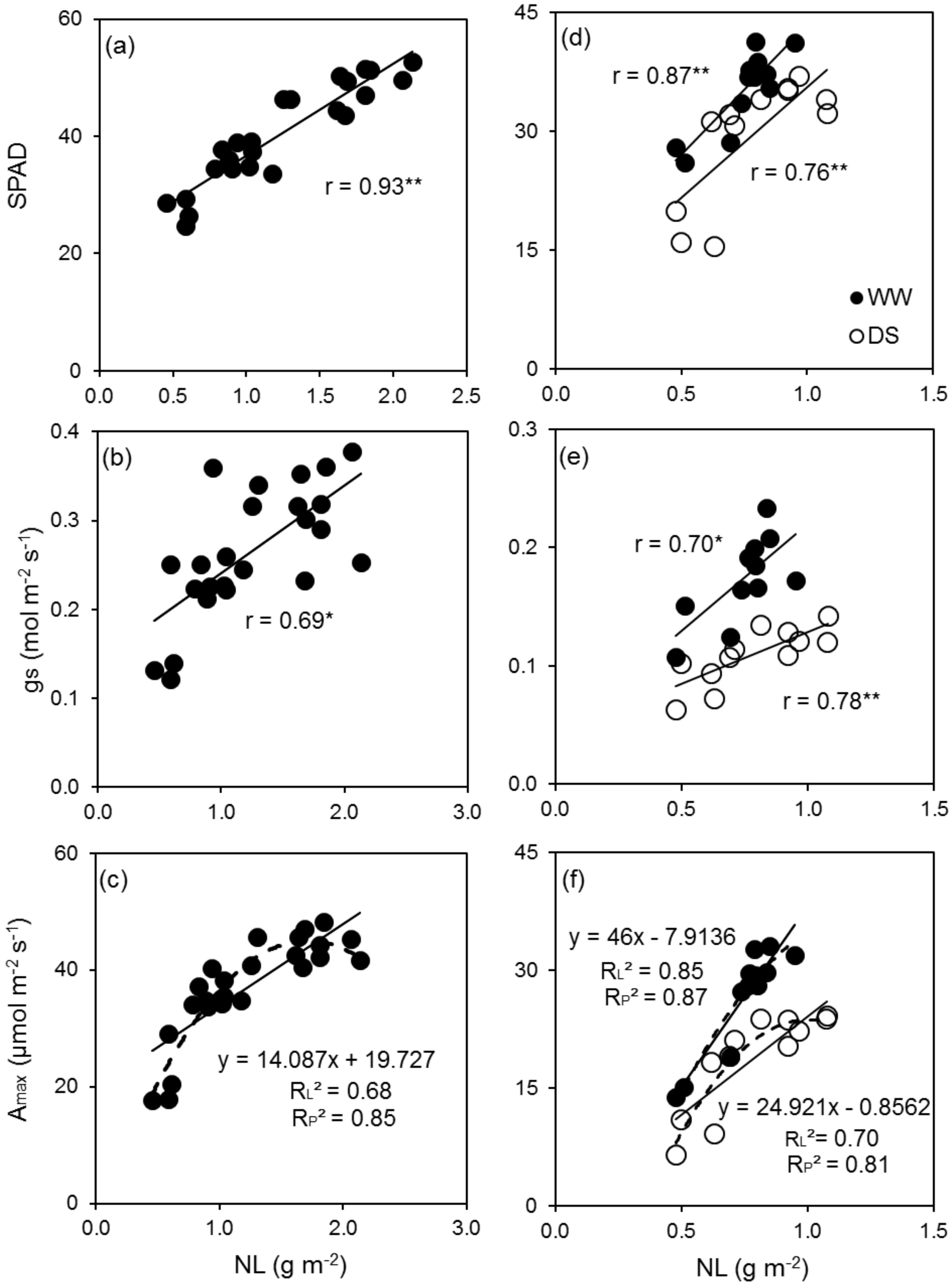

Figure 2.4. The correlation among SPAD, stomatal conductance (g_s), potential photosynthetic rate (A_{max}) and leaf nitrogen content (NL) at 60DAT (a, b, c) and at 120DAT (d, e, f) under well-watered (closed shape) and drought stress conditions (opened shape), respectively. R_L^2 and R_P^2 are R square values on the chart of linear and polynomial regression types, respectively.

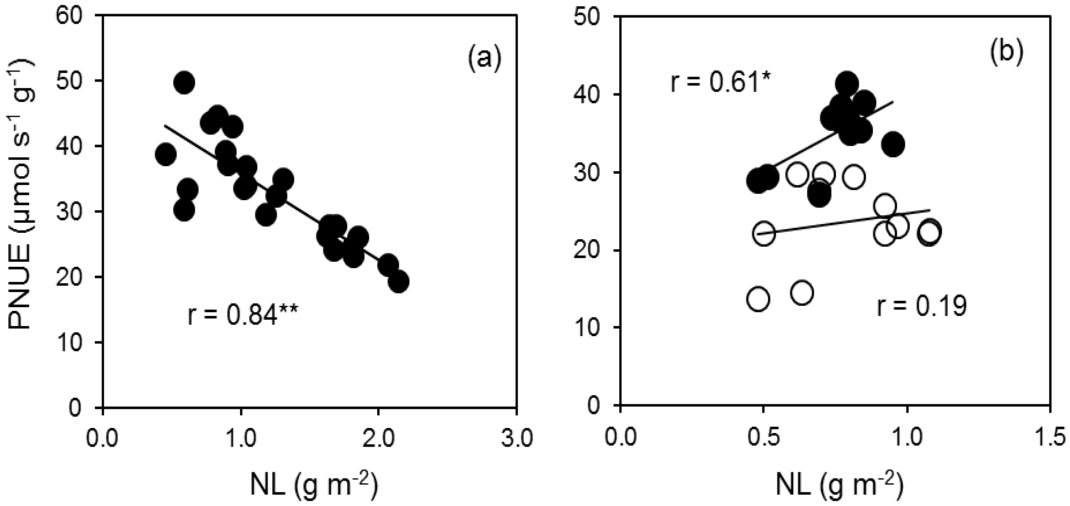

Figure 2.5. The correlation between specific leaf nitrogen content (NL) and photosynthetic nitrogen use efficiency (PNUE) at 60DAT (a); and at 120DAT (b) under well-watered (closed shape) and drought stress (opened shape) conditions.

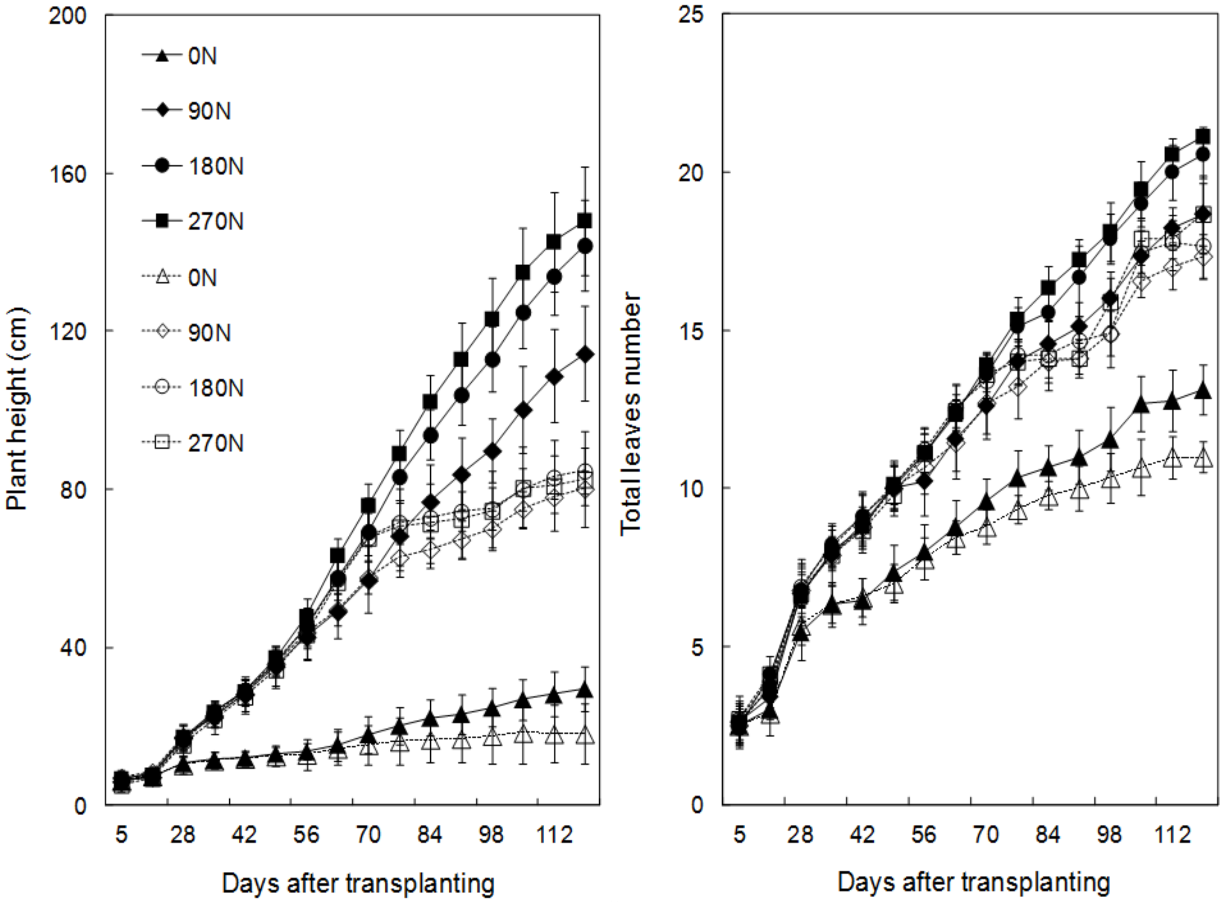

Figure 2.6. Plant height and total leaves number under well-watered (closed shapes) and drought stress (opened shapes) conditions with different nitrogen application levels.

Effects of drought and nitrogen application on agronomic traits of sugarcane

As can be seen from Figure 2.6, there were obvious differences in plant height and total leaves number of fertilized treatments with 0N treatment starting from 14DAT. Under the full irrigating condition, among fertilized treatments, the difference in plant height and total leaves number became clearly from 63DAT with higher values belonging to higher N supplied treatments. However, there were no significant differences in these values between 180N and 270N treatments. From 63DAT under the effect of drought stress, increasing rates of plant height and total leaves number were decreased especially from 77DAT they did not virtually increase when water stress became more severe.

Leaf area was significantly different among N application treatments (Figure 2.7). Actually, it augmented significantly when N level was changed from 0N to 180N, but not as up to 270N. Increasing N application just had effects on biomass traits under sufficient water supply. Under stress conditions, the increase of biomass was just in case of increasing N from 0N to 90N, after that biomass became similar, especially between higher N supplied treatments. Drought stress reduced noticeably leaf area, shoot, root and total biomass of sugarcane (Figure 2.7). Earlier studies demonstrated positive effects of N application as well as negative effects of drought stress on leaf area, and shoot, root and total biomass of sugarcane (Robertson et al., 1999; Ramesh, 2000; Kumara and Bandara et al., 2001; Cha-um and Kirdmanee et al., 2008; Saleem et al., 2012; Jangpromma et al., 2012; Oliveira et al., 2016). The positive significant correlations among photosynthetic traits (A_{max}, NL, and SPAD) with biomass traits under both normal and drought stress conditions (Table 2.2) suggested that higher N applications could support higher photosynthesis activities to accumulate more dry matter. On the other hand, according to Bell et al. (2014) early shoot and root vigor, and higher root length density could support the greater uptake of nitrate and yield in the soils with high N leaching potential. In this study, at higher doses of N application, the plants had a faster growth rate (higher plant height and the number of leaves) than lower ones (Figure 2.6). Furthermore, larger root with higher dry mass and number of roots (data not shown) also found in higher applied N treatments. It seemd to be that adding N was advance to create earlier a larger root system for absorbing more nutrient, or rather, N. Root dry weight had significant correlations with shoot dry weight and total biomass under both conditions (Table 2.2). The positive correlations among root dry weight with leaf, shoot dry weight and total biomass were also presented by Jangpromma et al. (2012) and Jackson et al. (2016). These findings raise a point of view that larger root system by adding N at early stage helps plant uptake more nutrient to create more shoot dry weight, as a result increasing total biomass under full irrigation as well as drought stress conditions.

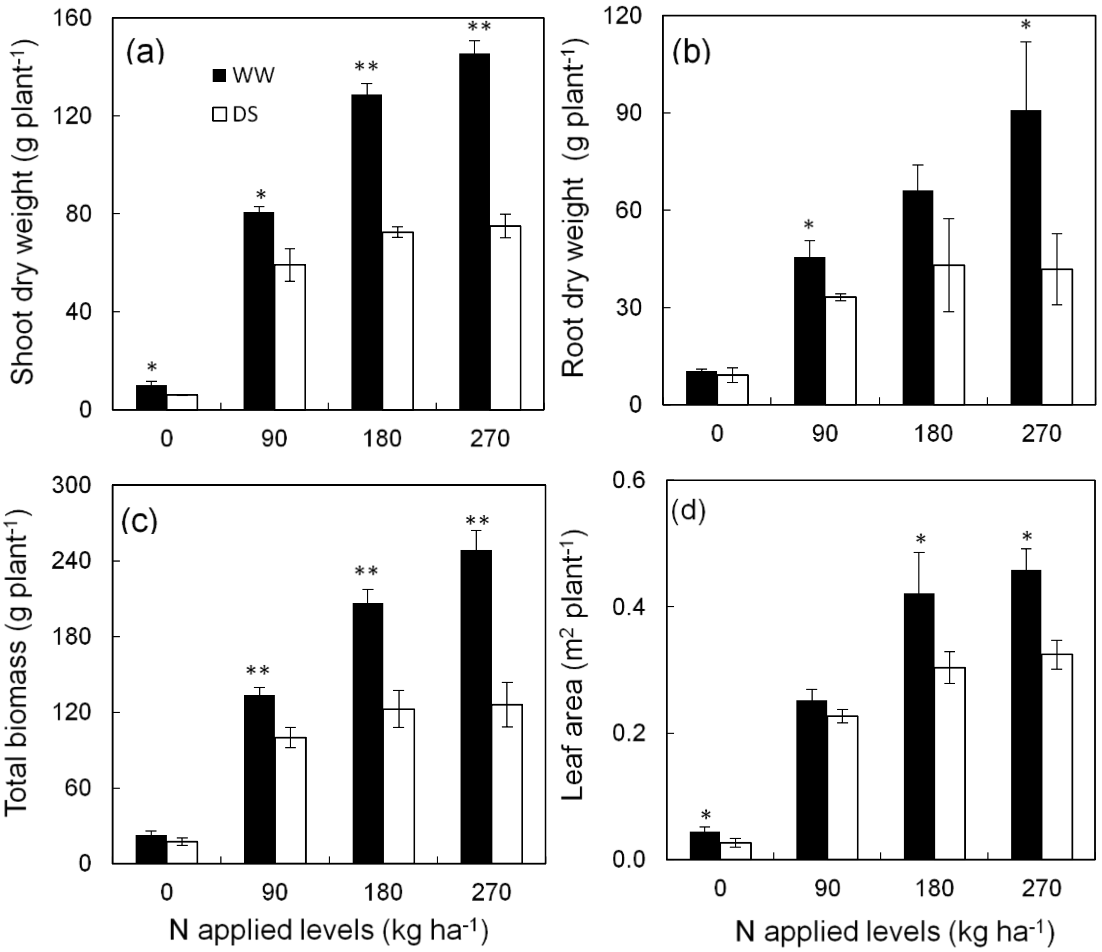

Figure 2.7. Shoot dry weight (a), root dry weight (b), total biomass (c) and leaf area (d) at 120DAT under well-watered (closed shape) and drought stress (opened shape) conditions with different nitrogen application levels.

Table 2.2. The correlations among photosynthetic traits and biomass production under well-watered and drought stress conditions

Investigated traits		Root dry weight	Shoot dry weight	Total biomass
Well-watered	A_{max}	0.85**	0.94**	0.92**
	Leaf N content	0.87**	0.84**	0.87**
	SPAD	0.89**	0.88**	0.90**
	Root dry weight	-	0.92**	0.97**
Drought stress	A_{max}	0.90**	0.97**	0.82**
	Leaf N content	0.73**	0.83**	0.82**
	SPAD	0.81**	0.96**	0.93**
	Root dry weight	-	0.89**	0.95**

** means significant at $P<0.01$.

There are several investigations in the relationship between NUE and drought tolerance ability of cereal crops. In winter wheat, NUE in a line with high drought tolerance was higher than that of the line with low drought stress tolerance (Fan and Li, 2001). In maize, due to having either high N uptake or N utilization efficiency, drought tolerant cultivars produced consistently higher yields (Kamara et al., 2014). In sweet sorghum, improved water use efficiency and NUEs under water stress may both contribute to the high degree of physiological acclimation to drought (Wang et al., 2014). In this study, the relationships among PNUE, BNUE, and DTI were shown in Figure 2.8 with strong positive coefficients (r= 0.90** and r= 0.99**, respectively). This finding highlighted that higher PNUE or BNUE could help plant have higher ability to tolerate to drought stress. However, further studies with more diversity of sugarcane varieties should be conducted to stronger support these findings. Furthermore, although higher N application levels had better performances (not significant especially under drought conditions), the lower levels with higher PNUE or BNUE could diminish the negative effects of drought stress. In the context of this study, a range of N application from 90 to 180 kg N ha^{-1} seems to be the appropriate amount to maintain acceptable growth under water deficit condition. However, to suggest an optimum amount for N application, a larger scale experiment under field conditions should be investigated in later studies.

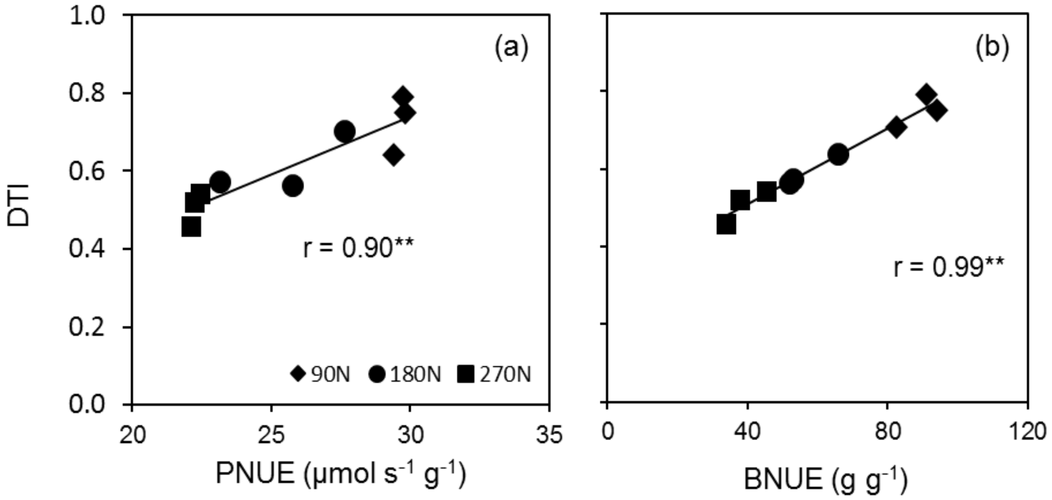

Figure 2.8. The correlations among drought-tolerant index (DTI) with photosynthetic nitrogen use efficiency (PNUE) (a), and biomass nitrogen use efficiency (BNUE) (b).

2.2. Effects of drought stress at early growth stage on the response of sugarcane to different nitrogen application

2.2.1. Introduction

Sugarcane is an important cash crop which has been grown widely in the tropical regions. However, most of the sugarcane production area is grown under the rain-fed condition, wherein drought stress is the main reason for low yield and low sugar quality. The early growth and mid-season are critical stages of drought stress sensitivity due to the high water requirement for sustainable growth in sugarcane (Zingaretti et al., 2012). Drought stress at early growth stage and mid-season reduce plant growth expressing in stunting, restriction of tillering which lead to vacant and low millable stalk, and finally cause yield loss in both cane yield and sugar yield. Although mid-season drought has the most drastic constraint on sugarcane yield, by cropping season management it is often arranged in the rainy season wherein the effect of drought stress is diminished. Hence, water stress during early growth stage becomes more frequently.

N involves in many critical processes such as plant growth, expansion of green leaves, and tiller or sucker production. Unfortunately, N is often used to promote sugarcane growth during early growth stage from tillering until early grand growth phase. Water deficit which often occurs in this stage could lead to insufficient in N supplement because of restricting ability in uptake and partitioning of N into the plant. Hence, the shortage in N source from water stress might result in the reductions of yield and sugar quality. Under normal irrigation condition, previous studies reported that yield and yield attributing characters were positively influenced by the increasing dose of N (Singh et al., 2000; Madhuri et al., 2011). Asokan et al. (2005) found the uptake of N increased with the increase of N application, but cane yield did not show any significant increase when N was applied over 100 kg ha^{-1}. These authors had the same point that different N application levels did not have a well-marked effect on juice quality. However, it still doubts in the effect of drought stress on the efficiency of applying N on sugarcane growth, yield, and sugar quality. Responses of sugarcane to N fertilization were not significantly affected by moisture stress during grand growth stage (Wiedenfeld, 2000), but different irrigation levels which were induced throughout the whole growing-season had strong effects on sugarcane yield and quality (Wiedenfeld, 1995). Previous studies documented that drought stress at early growth stage reduced plant height, stalk diameter, the number of tillers, leaf area, and total biomass, sucrose content, cane and sugar yield (Robertson et al., 1999; Zhao et al., 2010; Jangpromma et al., 2012; Barbosa et al., 2015). However, there is, still, no information about the efficiency of N application on sugarcane growth and sugar quality under the impact of drought stress at early growth stage. In this study, the response of sugarcane to different N application for growth, biomass yield and juice parameters were investigated under both well-watered and drought stress condition at early growth stage.

2.2.2. Materials and methods

From May to October 2016, the experiment was conducted under glasshouse conditions at the University of the Ryukyus, Okinawa, Japan (26°25’ N, 127°45’ E; altitude 126 m). The commercial sugarcane variety NiF8 (*Saccharum* spp.), which has high yield and high sucrose content but unstable under unfavorable conditions (Matsuoka, 2006), was used. The two months old seedlings were transplanted into Wagner pots 1/2000a filled with 10 kg substrate of Shimajiri Mahji red soil: sea sand: peat moss (1: 1: 1, v v^{-1}) at 12% of soil moisture content.

Experimental design

The experimental design was a split-plot in a randomized complete block design with 3 replications. The main factor treatment, four N applied levels at 0, 5.7, 11.4 and 17.1g ammonium sulfate pot^{-1} (equivalent to 0, 90, 180 and 270 kg N ha^{-1}, respectively), was assigned in subplots. The sub-factor treatment, two water regimes included well-watered (as the control treatment) and drought stress during the early growth stage was assigned in main plots. At well-watered treatment, soil moisture content was maintained at field capacity from transplanting until the end of the experiment. Meanwhile, at drought stress treatment, soil moisture content was kept at field capacity until 60DAT, then it was monitored at 1/3 available water for 60 days, then recovering at field capacity from 120DAT until the end of the experiment (160DAT).

During the experimental period, all tillers were removed immediately after germination to keep only mother stalk in the pot. At 7DAT, superphosphate at rate 5.2 g pot^{-1} was applied. Potassium chloride at a rate of 2.1 g pot^{-1} and ammonium sulfate were divided by 5 times at the ratio of 2: 3: 2: 3: 3 to fertilize at 7, 30, 60, 90 and 120DAT, respectively.

Data collection

At 120DAT (drought stress period) and 160DAT (recovery period), the sample plants were taken to determine the photosynthetic and agronomical parameters. However, in term of this part, I will show the data for photosynthetic and agronomical parameters at the recovery period and for sugar parameter at both periods. Another data for photosynthetic and biomass parameters at stress period was shown in Chapter 2.1.

Photosynthetic parameters

A_{max} and g_s were determined at the first fully expanded leaf of sample plant of each treatment in all replications by LI-6400 portable photosynthesis system (LI-COR, Lincoln, Nebraska, USA) equipped with a 2 x 3 cm^2 LED chamber between 900 to 1500 hours. Photon flux density, leaf temperature, and CO_2 concentration were set up at 2000 µmol m^{-2} s^{-1}, 31 ± 2°C and 400 ± 5 µmol mol^{-1}, respectively.

After photosynthesis measurement, SPAD values were recorded at the same positions using a SPAD meter (SPAD-502, Minolta, Japan). Then, the first full leaf sample was cut and dried at 80°C for 48hr to determine leaf dry weight. After grinding by TI-100 vibrating sample mill (CMT, Tokyo, Japan), 25 mg of dry leaf sample was taken to determine specific leaf N content (NL) using an N/C analyzer (NC-90A, Shimadzu, Japan).

Agronomical parameters

Before harvest, the growth parameters were determined. The plant height was measured from soil surface to blade joint of the first full leaf on the top of the plant. Stalk diameter was measured at the middle of inter-node on the stalk. The number of nodes per plant was also counted. Then, the whole plant leaf blades were cut to determine leaf area using LI-3100 leaf area meter (LI-COR, Lincoln, Nebraska, USA).

After removal of parts unnecessary for sugar refining, the millable stalk was weighted and squeezed. Juice weight was calculated by the difference of millable stalk and bagasse fresh weight. Then leaves, bagasse and remained parts were dried at 80°C for 48hr to determine to shoot dry weight. The root sample separated from above ground parts was cleaned by tap water, and then shoot root number was counted before drying at 80°C for 48hr to determine root dry weight. Total biomass was calculated by the sum of the shoot and root dry weight.

Then, biomass nitrogen use efficiency (BNUE) and biomass water use efficiency (BWUE) was calculated by the following:

BNUE = Total biomass/ amount of N applied;

BWUE = Total biomass/ total amount of water application.

Sugar parameters

After squeezing, juice samples after filtered by a sieve with a wire diameter of 50 µm were stored at -80°C until used for juice analysis to prevent deterioration in quality. After juice samples were completely melted, sugars components (sucrose and reducing sugar) concentrations were determined by high-pressure liquid chromatography (LC-10A, Shimazu); concentrations of major ions in sugar juice were determined by ion chromatography (ICS-1600, Thermo Fisher Scientific). Sugar yield was calculated by the product of juice weight and total sugar concentrations.

Data 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 ion concentrations with agronomical and sugar quality were calculated to assess the relationships.

2.2.3. Results

Effect of drought stress and nitrogen application on photosynthesis

As can be seen from Table 2.3, drought stress (after re-watering) did not affect negatively photosynthetic traits, including A_{max}, g_s, SPAD and NL. Under the well-watered condition, these traits were even lower (unnoticeable) than those under stress condition. Nevertheless, the remarkable decrease in leaf area was recorded. On the other hand, varying N application levels brought out the differences in all photosynthetic traits and leaf area. Actually, there were upward trends of these traits when N supplement was increased. However, the significant increase was just found when N was added from 0N up to 90N. For higher N supplement, the significances were recorded just in case of A_{max}, leaf area and NL under well-watered condition until when adding N up to 180N.

Table 2.3. Potential photosynthetic rate (A_{max}), stomatal conductance (g_s), SPAD, specific leaf nitrogen content (NL) and leaf area under different water regimes and nitrogen application levels

Water Regimes	N levels	A_{max} (μmol m^{-2} s^{-1})	g_s (mol m^{-2} s^{-1})	SPAD	NL (g m^{-2})	Leaf area (m^2)
Well-Watered	0N	17.8^b	0.10^b	31.9^b	0.64^b	0.05^c
	90N	21.2^b	0.17^{ab}	34.5^{ab}	0.72^b	0.14^b
	180N	28.1^a	0.23^a	40.0^a	0.95^a	0.24^a
	270N	28.9^a	0.20^{ab}	40.6^a	0.96^a	0.25^a
	Average	24.0	0.18	36.8	0.82	0.17
Drought Stress	0N	15.8^b	0.13^b	30.0^b	0.78^b	0.03^b
	90N	24.4^{ab}	0.20^a	37.9^{ab}	0.96^{ab}	0.15^a
	180N	31.0^a	0.23^a	43.3^a	1.06^{ab}	0.16^a
	270N	31.9^a	0.23^a	46.5^a	1.23^a	0.19^a
	Average	25.8	0.20	39.4	1.01	0.13
Water regimes (W)		Ns	ns	ns	ns	*
N levels (N)		**	**	**	**	**
W*N		Ns	ns	ns	ns	*

ns, * and ** mean non-significant, significant at $P < 0.05$ and $P < 0.01$, respectively. Different letters in the same column show significance among N applied levels at $P < 0.05$ by Turkey.

Effect of drought stress and nitrogen application on sugarcane growth

Effects of drought stress and N application on plant height, stalk diameter, the number of nodes and shoot root per plant were shown in Figure 2.9. The result revealed that all of these traits were reduced significantly under drought stress conditions. The significant differences, which higher values for all traits were the result of higher doses of N application, were also shown. However, the differences were clearer under sufficient water supplement condition. Under this condition, higher values were found when increasing N applied levels from 0N to 180N. Meanwhile, under drought condition, the significant difference was found between fertilized treatments with non-fertilized treatment (0N) only.

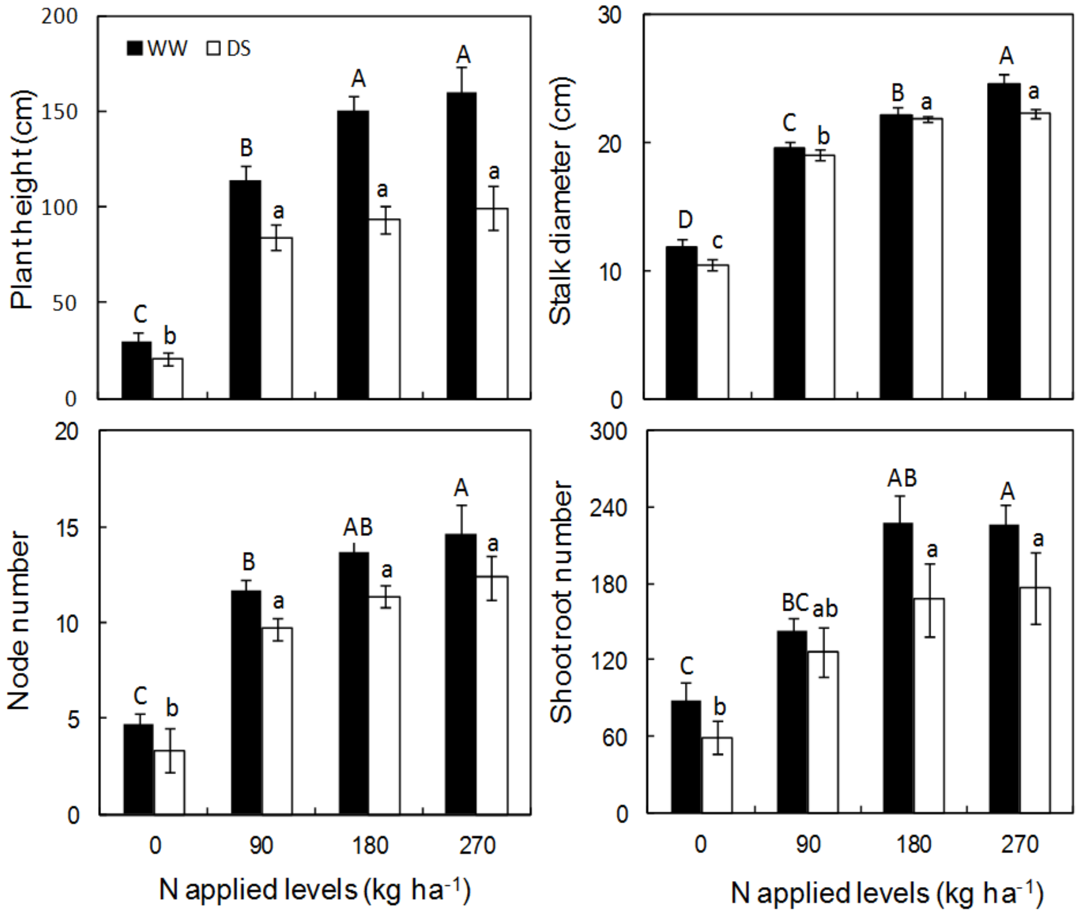

Figure 2.9. Plant height, plant diameter, node number and shoot root number/plant under different water regimes and nitrogen application levels.

WW and DS: well-watered and drought stress treatment, respectively; different capital and lowercase letters show significance among N applied levels within well-watered and drought stress condition at $P < 0.05$ by Turkey, respectively.

The result in Table 2.4 indicated that biomass traits, BNUE as well as BWUE of sugarcane cv. NiF8 were declined under water stress condition. Applying N induced increase of biomass traits and BWUE. However, under drought conditions, the significant increase was found in shoot dry weight when N was applied up to 180N, but just up to 90N for another. Under the well-watered condition, more obviously, higher values in root dry weight and total dry matter accumulation corresponded with higher in N application at all rates, whereas shoot dry weight and BWUE increased not significantly when N applied was from 180N to 270N. In the opposite way, there were reductions in BNUE when N was dressed from 90N to 180N, and to 270N with percentage reduction of 20.1 and 36.8%, respectively. The reductions were clearer under effects of drought stress, wherein BNUE decreased by 37.0 and 56.1% when increasing N application does from 90N to 180N, and to 270N, respectively.

Table 2.4. Shoot and root dry weight (DW), total biomass, biomass nitrogen use efficiency (BNUE) and biomass water use efficiency (BWUE) under different water regimes and nitrogen application levels

Water regimes	N levels	Shoot DW (g plant^{-1})	Root DW (g plant^{-1})	Biomass (g plant^{-1})	BNUE (g g^{-1})	BWUE (g g^{-1})
Well-watered	0N	13.1^{c}	9.7^{d}	22.8^{d}	-	0.6^{c}
	90N	99.5^{b}	42.0^{c}	141.5^{c}	99.2^{a}	2.1^{b}
	180N	154.1^{a}	58.3^{b}	212.4^{b}	79.2^{b}	2.5^{a}
	270N	172.0^{a}	76.3^{a}	248.3^{a}	62.8^{c}	2.6^{a}
	Average	109.7	46.6	156.3	16.9	2.0
Drought stress	0N	7.2^{c}	7.2^{b}	14.4^{c}	-	0.5^{b}
	90N	70.7^{b}	42.6^{a}	113.3^{b}	82.6^{a}	2.2^{a}
	180N	96.0^{a}	42.1^{a}	138.1^{ab}	51.7^{b}	2.2^{a}
	270N	107.0^{a}	37.5^{a}	144.5^{a}	36.2^{b}	2.2^{a}
	Average	70.2	32.3	102.6	11.9	1.8
Water regimes (W)		**	**	**	**	*
N levels (N)		**	**	**	**	**
W*N		**	**	**	ns	ns

ns, * and ** mean non-significant, significant at $P < 0.05$ and $P < 0.01$, respectively. Different letters in the same column show significance among N applied levels at $P < 0.05$ by Turkey.

Effect of drought stress and nitrogen application on sugar yield and sugar quality

Drought stress reduced noticeably juice weight and sugar yield of sugarcane but did not have a clear effect on total sugar content (Figure 2.10). In fact, juice weight and sugar yield under stress condition were as half as those under control conditions at both drought stress and recovery periods. Sucrose, reducing sugar and total sugar contents under well-watered condition were higher than those under stress condition (excepting for sucrose, total sugar content at 0N treatment during the drought period). However, the significant differences between water regimes were recorded in sucrose and total sugar content in unfertilized treatment only.

There were significant differences in total sugar content, juice weight and sugar yield among N treatments (Figure 2.10). Actually, reducing sugar content increased following increasing of N applied levels. Sucrose and total sugar content increased rapidly to reach the peaks at 90N in well-watered and drought stress conditions, respectively. Then, they slightly dropped at the higher rates of N application. Juice weight and sugar yield rose following the change of N in the range of 0N to 180N, but at higher N application rate, the increase was not significant.

Because of that the concentrations of F^-, NO_2^- and NO_3^- were very low to be able to evaluate the effects of N and drought stress on accumulation of these ions in sugarcane juice, this study just shows data of Cl^-, SO_4^{2-}, PO_4^{3-}, NH_4^+, K^+, Ca^{2+}, Mg^{2+} and Na^+ (Figure 2.11a, b). The concentrations of all investigated anions (excepting for PO_4^{3-} with a reduction) and cations increased under drought condition. However, the significant increases were just found in the concentration of Cl^-, NH_4^+, and K^+. Change in N application affected concentrations of all ions excepting for SO_4^{2-}. While concentrations of NH_4^+ increased, the concentrations of anions and other cations had downward trends when increasing N levels from 0N to 270N. However, the obvious tendency was found in K+ concentration only.

Across water regimes and N levels, all of the ion concentrations (excepting for NH_4^+ and SO_4^{2-}) had negative correlations with stalk diameter, plant height, total sugar content, juice weight and sugar yield (Table 2.5). The concentration of Cl^- had positive significant correlations with Ca^{2+} and K^+. On the contrary, the significant correlations were found only between the concentration of SO_4^{2-} and Mg^{2+}, and between those of PO_4^{3-} with K^+ and Na^+. There was no relationship between concentrations of SO_4^{2-} and Cl^-, but a positive relationship was found between concentrations of PO_4^{3-} and Cl^-. Among cations, all other had positive correlations with each other, but the concentration of NH_4^+ had significant positive correlations with that of Mg^{2+} only (Table 2.6).

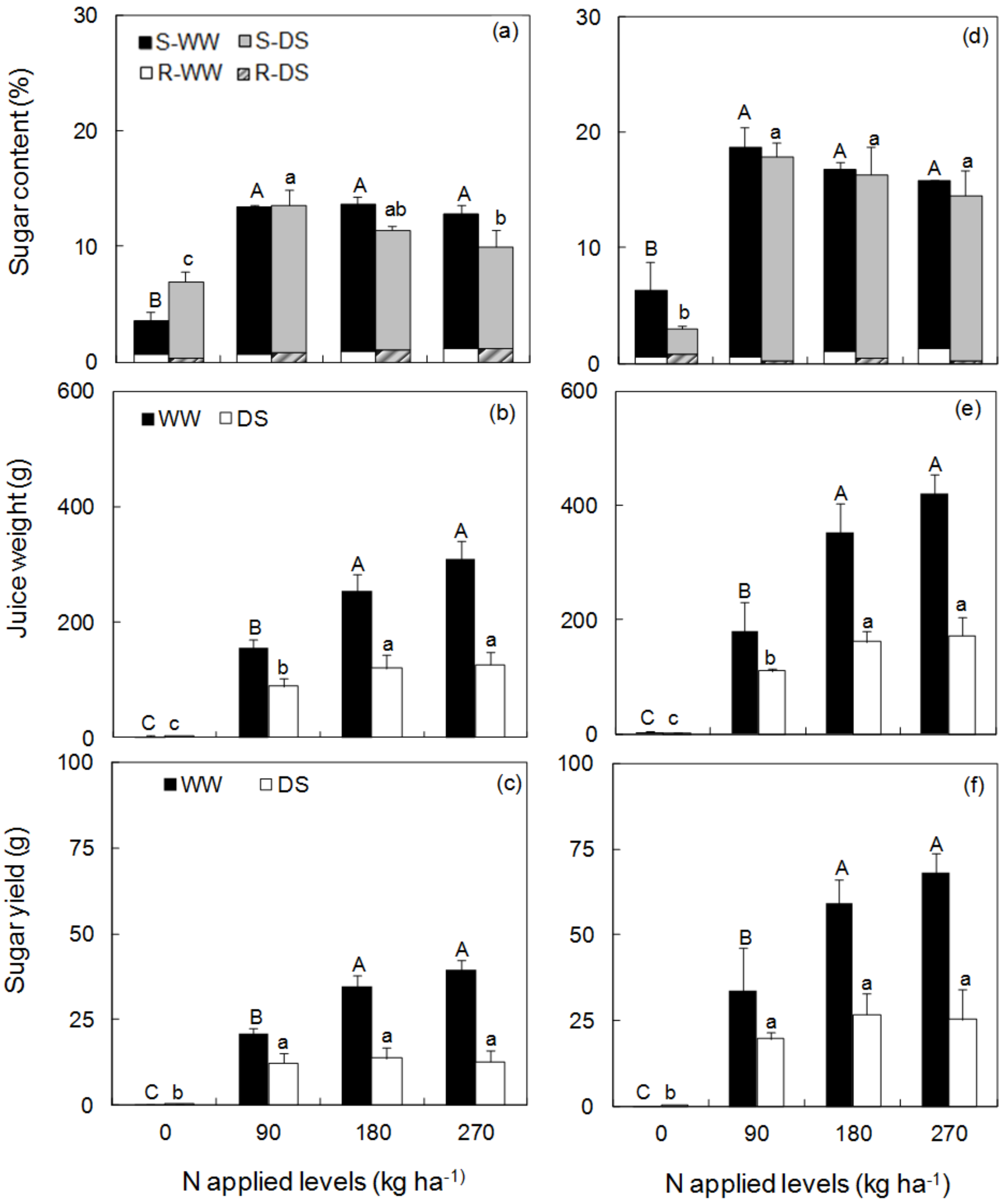

Figure 2.10. Sugar content, juice weight and sugar yield at drought (a, b, c) and recovery (d, e, f) periods under different water regimes and nitrogen application levels

S-WW and S-DS: sucrose content of drought stress and well-watered treatment, respectively; R-WW and R-DS: reducing sugar content of well-watered and drought stress treatment, respectively; WW and DS: well-watered and drought stress treatment, respectively; different capital and lowercase letters show significance among N applied levels within well-watered and drought stress conditions at $P < 0.05$ by Turkey, respectively.

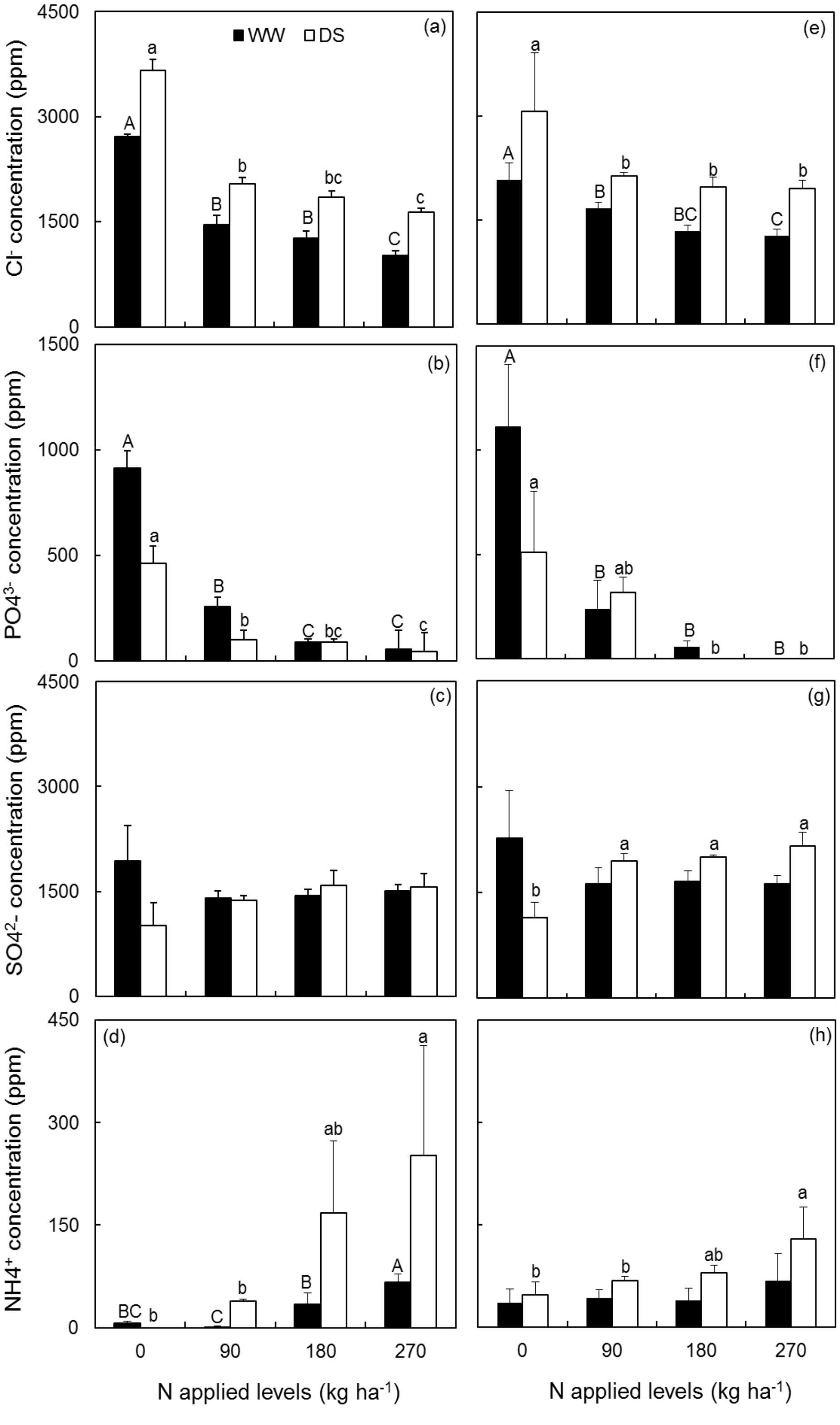

Figure 2.11a. Cl^-, $PO_4^{3-,}$ SO_4^{2-} and NH_4^+ concentrations at drought (a, b, c, d) and recovery (e, f, g, h) periods under different water regimes and nitrogen application levels

WW and DS: well-watered and drought stress treatment, respectively; different capital and lowercase letters show significance among N applied levels within well-watered and drought stress conditions at $P < 0.05$ by Turkey, respectively.

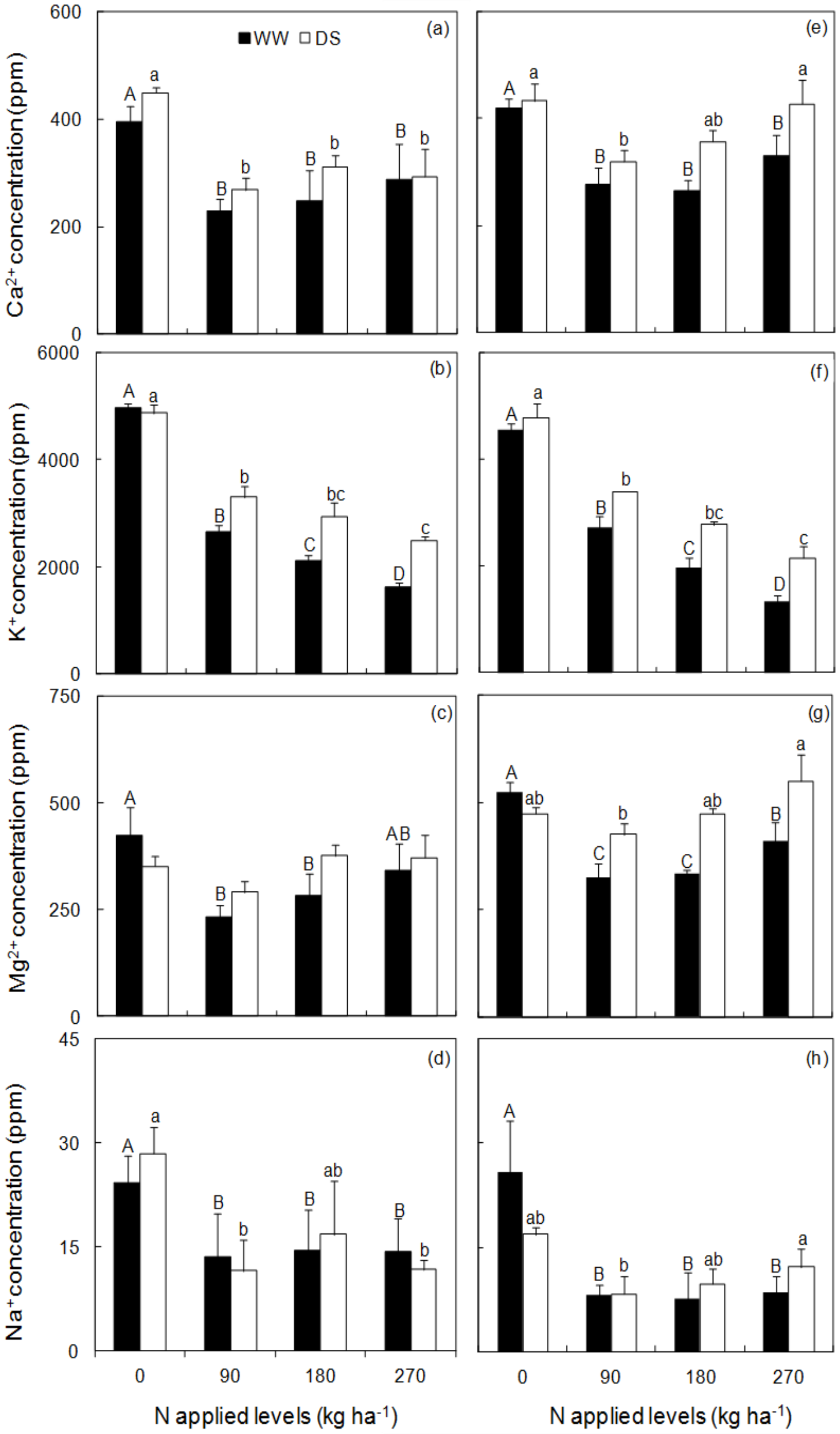

Figure 2.11b. Ca$^{2+,}$ K^{+}, Mg^{2+}, and Na^{+} concentrations at drought (a, b, c, d) and recovery (e, f, g, h) periods under different water regimes and nitrogen application levels

WW and DS: well-watered and drought stress treatment, respectively; different capital and lowercase letters show significance among N applied levels within well-watered and drought stress condition at $P < 0.05$ by Turkey, respectively.

Table 2.5. Correlations among ion contents in juice and plant height, plant diameter, total sugar content, juice weight and sugar yield across water regimes and nitrogen application levels

Parameters	Plant height	Stalk diameter	Sugar content	Juice weight	Sugar yield
Cl^-	-0.78**	-0.72**	-0.66**	-0.75**	-0.74**
PO_4^{3-}	-0.82**	-0.71**	-0.63**	-0.68**	-0.65**
SO_4^{2-}	-0.03	0.15	0.21	-0.06	-0.17
NH_4^+	0.04	0.33	0.13	-0.04	-0.10
Ca^{2+}	-0.71**	-0.52**	-0.74**	-0.62**	-0.66**
K^+	-0.95**	-0.96**	-0.78**	-0.92**	-0.90**
Mg^{2+}	-0.59**	-0.32	-0.53**	-0.54**	-0.59**
Na^+	-0.73**	-0.74**	-0.77**	-0.63**	-0.66**

* and ** mean significant at $P < 0.05$ and $P < 0.01$, respectively.

Table 2.6. Correlations among the anion and cation contents in juice across water regimes and nitrogen application levels

Parameters	Cl^-	PO_4^{3-}	SO_4^{2-}	NH_4^+	Ca^{2+}	K^+	Mg^{2+}
Cl^-	-	-	-	-	-	-	-
PO_4^{3-}	0.50*	-	-	-	-	-	-
SO_4^{2-}	-0.08	0.28	-	-	-	-	-
NH_4^+	0.09	-0.37	0.38	-	-	-	-
Ca^{2+}	0.51*	0.37	0.18	0.38	-	-	-
K^+	0.73**	0.77**	-0.06	-0.28	0.53**	-	-
Mg^{2+}	0.40	0.29	0.44*	0.54**	0.93**	0.38	-
Na^+	0.39	0.83**	0.29	-0.17	0.67**	0.69**	0.60**

* and ** mean significant at $P < 0.05$ and $P < 0.01$, respectively.

2.2.4. Discussion

Effect of drought stress on photosynthesis, growth and sugar parameters

Previous studies found the reductions in photosynthetic traits when plant subjected to drought stress (Du et al., 1996; Jangpromma et al., 2010; Graça et al., 2010; Barbosa et al., 2015). Actually, in Chapter 2.1, photosynthetic parameters were recorded at 120DAT (drought stress period) which also showed A_{max}, gs, and SPAD were significantly reduced under the effect of water deficit during the stress period. However, photosynthesis of C_4 plant is highly sensitive to water deficit (Ghannoum, 2009), it is easy to reduce when soil moisture content decrease, but also easy to recover when re-watering. In this study, the photosynthetic traits were investigated in the recovery period, therefore, under water stress conditions somewhat were higher than their counterparts under well-watered conditions. Mederios et al. (2013) found similar results in recovery of photosynthetic rates of both investigated sugarcane cultivars. Drought stress plant, after re-watering, might be stimulated to uptake more water which following by nutrient, or rather, N because of increasing water potential in plant tissue (Nonami, 1998). Because of positive correlations among leaf N content, chlorophyll content and photosynthetic rate (Kawamitsu et al., 1999; Ilkaee et al., 2016; Chapter 2.1), higher N accumulated into leaves of plants in drought stress treatment at recovery period might support higher leaf chlorophyll content (SPAD) and photosynthetic rate compared to those in control treatments (Table 2.3).

The results showed that drought stress at early growth stage had a significant effect on almost growth parameters. Similarly, by testing the response of sugarcane cultivars to a short drought period from 90 to 100 day after planting and recovery at 10 days later, Jangpromma et al. (2012) also found that drought stress significantly reduced stalk diameter, root dry weight as well as total biomass production. Zhao et al. (2010) and Barbosa et al. (2015) found the same effect of early drought stress on the number of leaves, green leaves area, plant height, stalk diameter, the number of tillers, and shoot biomass. These are consistent with the effects of drought stress under field conditions which reduced stalk height, stalk diameter (Begum and Islam, 2012), total dry matter (Basnayake et al., 2012) and cane yield (Basnayake et al., 2012; Begum and Islam, 2012). Similarly, drought stress at initial, mid-season and late-season stages reduced stalk height, and just late-season had no effect on cane and sugar yield (Ethan et al. 2016). Robertson et al. (1999) also reported that early drought stress at the tillering stage decreased leaf area index, millable stalk biomass as well as total biomass and cane yield. Drought stress, which diminished root growth in both number and root mass, may be the reason the reduction in turgor pressure, the interruption of water flow from the xylem to the surrounding elongating cells, and a slowing down of the growth process, particularly in terms of cell elongation and cell volume (Nonami 1998; Larcher 2003, Jaiphong et al., 2016). As the result, it caused reductions in stalk growth which directly relate to shoot dry matter as well as whole plant biomass.

Total sugar content and sugar yield were decreased after going through water stress period. Although the decline of sugar content was not significant, the strong effect of drought stress on stalk length and stalk diameter led to a remarkable reduction in juice weight, and as a result, sugar yield was decreased significantly even water was re-supplied. This matched the finding that quality traits were less sensitive to drought stress than yield component parameters (Hemaprabha et al., 2004). Hemaprabha et al. (2004) also found the reduction in sugar quality parameters including Brix, sucrose content, purity and Commercial Cane Sugar (CCS) when plant subjected to drought stress at formative and grand growth stages. Similarly, Singh and Naidu, (1985), and Robertson et al. (1999) also found that sucrose content, CCS and sucrose yield were reduced under the effect of water deficit at the formative phase.

Effect of nitrogen application on photosynthetic, growth and sugar parameters

Increasing N application levels support higher photosynthetic activities and agronomical traits, especially under well-watered conditions. Under drought condition, the clear differences in these traits were only found between fertilized treatments and non-fertilized treatments. On the other hand, sugar contents were not significantly different among fertilized treatments, but those were still higher than that in non-fertilized treatment. Under normal condition, Kawamitsu et al. (1999) also found the similar tendency in plant height, leaf N content, SPAD, the photosynthetic rate at 110DAT when concentrations of N in supplied solution were changed from 0 to 2N. N fertilization increased the production of aboveground (leaves and stalk) and belowground (roots) parts, and the whole plant dry-matter (Bologna-Campbell et al., 2012). Similarly, under field conditions, previous studies agreed that yield and yield attributing characters were positively influenced by the increased dose of N than normal recommended dose, but levels of N content did not affect the quality parameters of sugar juice (Singh et al., 2000; Madhuri et al., 2011; Bologna-Campbell et al., 2012; Shekinah et al., 2012).

This study agreed with Thorburn et al. (2014) that NUE reduced when the dose of N application was increased. Under drought stress, wherein, the shortage of water supplement which restricted uptake of nutrient, including N (Lakshmanan and Robinson, 2014) might lessen the effect of using N to plant growth. Therefore, the reduction of NUE, especially at higher N applied treatments were clearer when plant subjected to drought stress. The previous study reported that N, from fertilizer application, which was mostly accumulated into leaf and root (Millard and Mackerron, 1986), exerts an osmotic effect, which results in better water absorption and water use efficiency (Kawamitsu et al., 1999). This explained better BWUEs were found at higher N applied treatments compared to 0N treatment. Although, the negative influence of water deficit on plant growth led to reductions in BWUE, and BWUE becoming similar among fertilized treatments, they were still significantly higher than BWUE of 0N treatment.

Effect of drought stress and nitrogen application on ion concentration in sugar juice

Interestingly, the effects of N levels on the concentration of NH_4^+ had the same tendency with NL. Tamaki and Kawamitsu (1998) also found increases of NH_4^+ concentration in leaf blade and stalk when N applied levels were increased. Nevertheless, the concentration of another ion, especially K^+ and Cl^- had downward trends when increasing N applied levels. The concentrations of all ions (excepting for PO_4^{3-}) in drought stress treatment were higher than those in control treatment. The high concentration of ion accumulated in plant organs could be an osmotic adjustment when plant subjects to drought stress. Rahman et al. (1971) reported that total N, K^+, Ca^{2+}, Mg^{2+}, Na^+, and Cl^- increased, but phosphorus decreased under soil moisture deficiency. Similarly, the increases were reported in the uptake of potassium and calcium in maize (Tanguilig et al., 1987), and in the uptake of potassium in drought-tolerant wheat varieties (Sinha, 1978). However, despite the accumulation of ions allowing osmotic adjustment to maintain growth under low water supplement conditions, plant growth may still be inhibited by stress because osmotic adjustment may not be sufficiently rapid to compensate for growth (Akinci and Losel, 2012). On the other hand, the osmotic adjustment which occurs because dry mass normally used to synthesize new cells instead accumulates in the cells as a solute or is deposited in fewer or smaller cells (Kramer and Boyer, 1995). It might demonstrate the negative correlation among plant height and stalk diameter with the concentrations of sugar juice ions (Table 2.5).

Thangavelu et al. (2003) found juice sodium had positive associations with potassium, calcium, magnesium, sulfate, chloride and negative associations with sucrose content, purity, and CCS. They also reported that when juice sodium was low, concentrations of other nutrient elements in juice were also low, which is a favorable situation for better sugar production. Gupta and Prasad (1971) also emphasized the necessity of applying N to avoid adverse effects of phosphorus, potassium, calcium, magnesium, sulfate, sodium and silica on juice quality (Thangvalu et al., 2003). From this study’s result (Table 2.5), increasing concentration of ions such as Cl^-, PO_4^{3-}, K^+, Ca^{2+}, Mg^{2+}, and Na^+ could be considered to have an influence on sugar yield and sugar quality. Watanabe et al. (2016) also reported that concentrations of K^+ and Cl^-, the most abundant ions in sugar juice, had a positive correlation. In this study, the positive correlations were also found among the concentration of Cl^- with those of cation ions, especially K^+ (r=0.73**). The accumulation of this couple of ions (K^+ and Cl^-) could be the reason for a reduction in sucrose content.

In conclusion, photosynthesis of sugarcane could recover, but agronomical and biomass traits could not from drought stress at early growth stage. Applying N had a beneficial effect on sugarcane growth as well as sugar yield and quality. Higher N application could support a higher photosynthetic rate to accumulate more dry matter with better sugar yield stored in sugarcane stalk. However, the efficiency of using N was declined under the disturbance of water deficit. On the other hand, despite at the high N dose (270N), sugarcane performed well under the well-watered condition with the highest biomass and sugar yield, but not significantly better than at medium dose (180N) did. Even under drought stress condition, the sugar content and sugar yield in high N treatment were somewhat lower than those in the medium dose were. Thus, under unpredictable stress, to maintain an acceptable growth and yield, 180 kg N ha^{-1} seems to be the appropriate N applied dose. However, the investigation was on the small scale with the artificial drought stress during sugar accumulating period, it had better conduct the further studies under field condition until harvesting stage to get a stronger suggestion for an optimum amount for N application.

CHAPTER III

NITROGEN USE EFFICIENCY OF SUGARCANE VARIETY UNDER DROUGHT STRESS AT EARLY GROWTH STAGE

3.1. Introduction

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 (Zuurbier and Vooren, 2008; Ehara et al., 1994). However, to create the optimum production, during its life cycle sugarcane requires a huge water amount with an annual rainfall of at least 1,500-2,000mm (FAO, 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 N are often fertilized to promote sugarcane growth. Drought stress, therefore, at first reduces N uptake (Silva et al., 2017), then affects the assimilation and remobilization processes of N by restricting enzyme activity e.g. nitrate reductase (Abayomi, 2001), and results in the decline of NUE.

Previous studies found that there were positive associations among 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 and Edgecombe, 2015). Moreover, Ranjith and Meizer (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 (Chapter 2.1). 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.

3.2. 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 2 blocks:

i) Block 1- five commercial sugarcane varieties, including NiF3, Ni9, Ni17, Ni21 and Ni22 (Table 1) at well-watered 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 well-watered (WW) and water stress (DS) for 60 days from 60DAT to 120DAT were assigned in the main plots. The five above varieties were designed in the subplots.

Table 3.1. 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 and Amami regions
Ni21	Medium/ slight wide	Slight long and thick, numbers: small	High	Kume island
Ni22	Medium/medium	Slight long and thick, numbers: large	High	Tanegashima and Amami regions

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

The two-month-old seedlings were transplanted into Wagner pots (1/2,000 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 x 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 $Ca(NO_3)_2 \cdot 4H_2O$, 4mM KNO_3, 2mM KH_2PO_4, 2mM $MgSO_4 \cdot 7H_2O$, 25µM H_3BO_3, 10µM $MnSO_4 \cdot 5H_2O$, 2µM $ZnSO_4 \cdot 7H_2O$, 0.5µM $CuSO_4 \cdot 5H_2O$, and 0.1mM $C_{10}H_{12}FeN_2NaO_8 \cdot 3H_2O$ (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) set up at 10 cm of depth. For well-watered 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 well-watered treatment until 60DAT. 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 two-week interval.

In 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. potential 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 x 3 cm LED chamber between 0900 to 1500. Photon flux density, leaf temperature, and CO_2 concentration were set up at PFD of 2000 µmol m^{-2} s^{-1}, 31 ± 2°C, 400 ± 5 µmol mol^{-1}, respectively. 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 48hr to determine partial dry weights.

In block 2: From two days before finishing the experiment, the first fully expanded leaves of 3 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 48hr to determine dry weight. At 120DAT when drought stress treatment was 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 48hr to determine partial dry weights. After that, the first leaf and other leaves, stalk, and the root of the sample plants was 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 N 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 (BNUE) was calculated by following formula:

BNUE (g g^{-1}) = total dry weight/ total N applied amount;

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

NL (g m^{-2}) = measured N content x leaf dry weight/leaf area;

PNUE (µmol s^{-1} g^{-1}) = A_{max}/NL;

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

TNU (g) = TN x total dry weight;

TNUE (g g^{-1}) = total dry weight/ TNU;

Drought tolerant index (DTI) was determined as follows:

DTI = dry weight under stress condition/ dry weight under well-watered 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 drought tolerant index were calculated to assess the relationships.

3.3. Results

Meteorological conditions and volume soil moisture in the experimental site were shown in Figure 3.1. 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. The soil moisture content of well-watered 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.

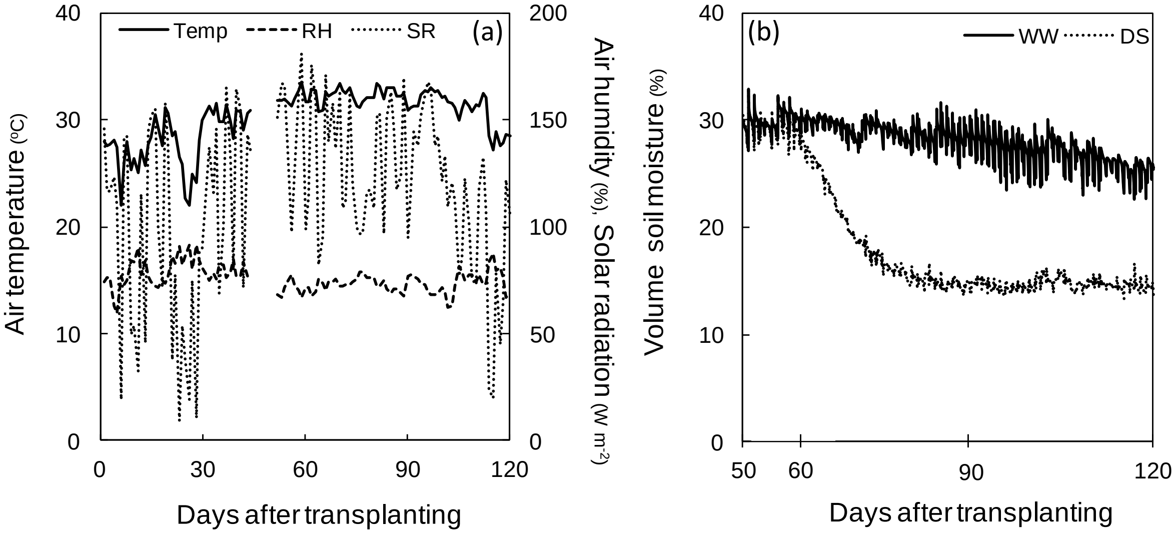

Figure 3.1. Weather conditions and soil moisture content during the experimental period

Temp, RH, and SR: daily average air temperature, air humidity, and solar radiation; WW: well-watered, DS: water stress.

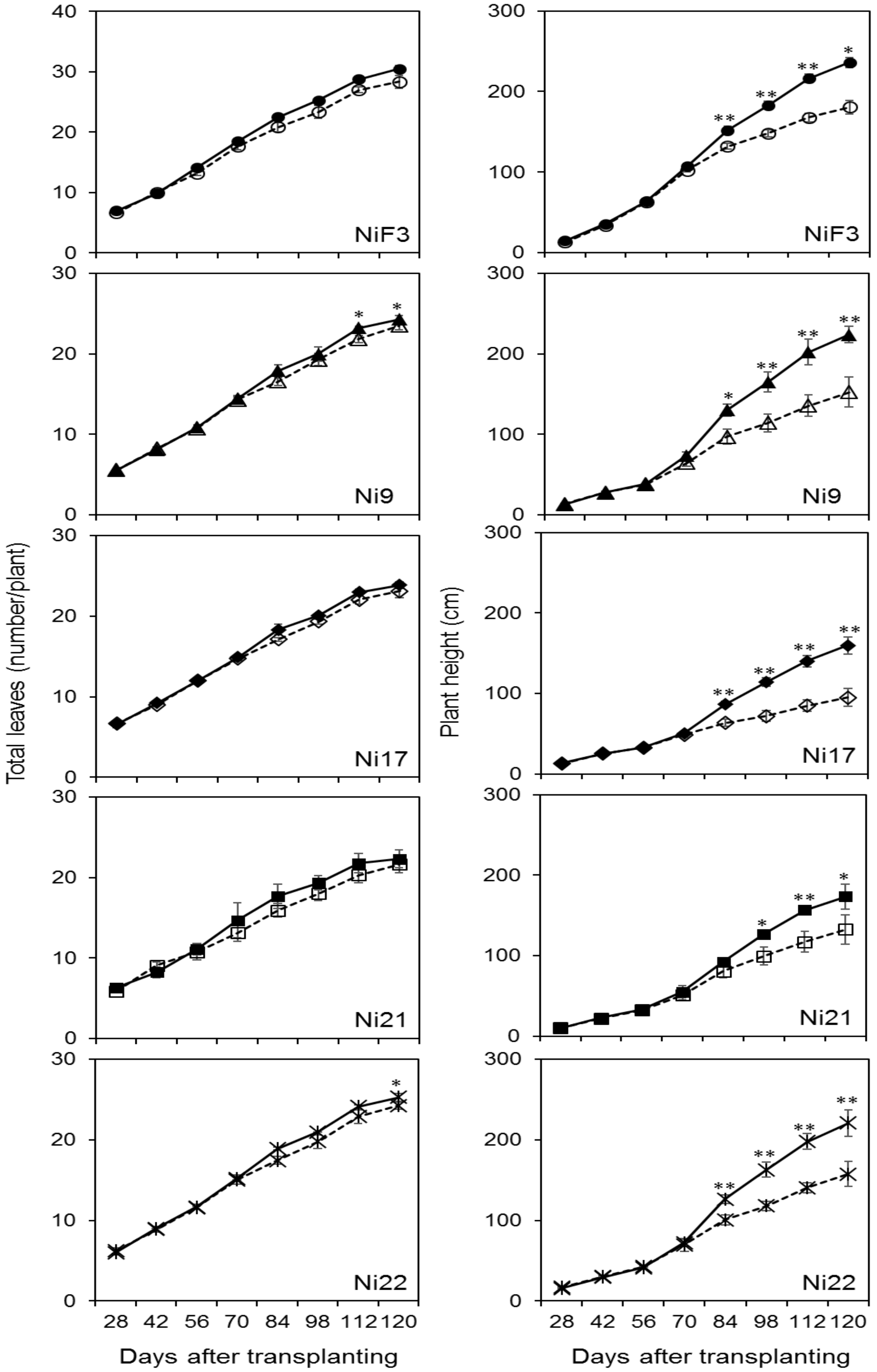

Figure 3.2. Total leaves number and plant height growth of sugarcane varieties under well-watered (filled symbols, solid line) and water stress conditions (non-filled symbols, dot line), respectively.

* and ** mean significant between well-watered and water stress treatments at $P < 0.05$ and $P < 0.01$, respectively.

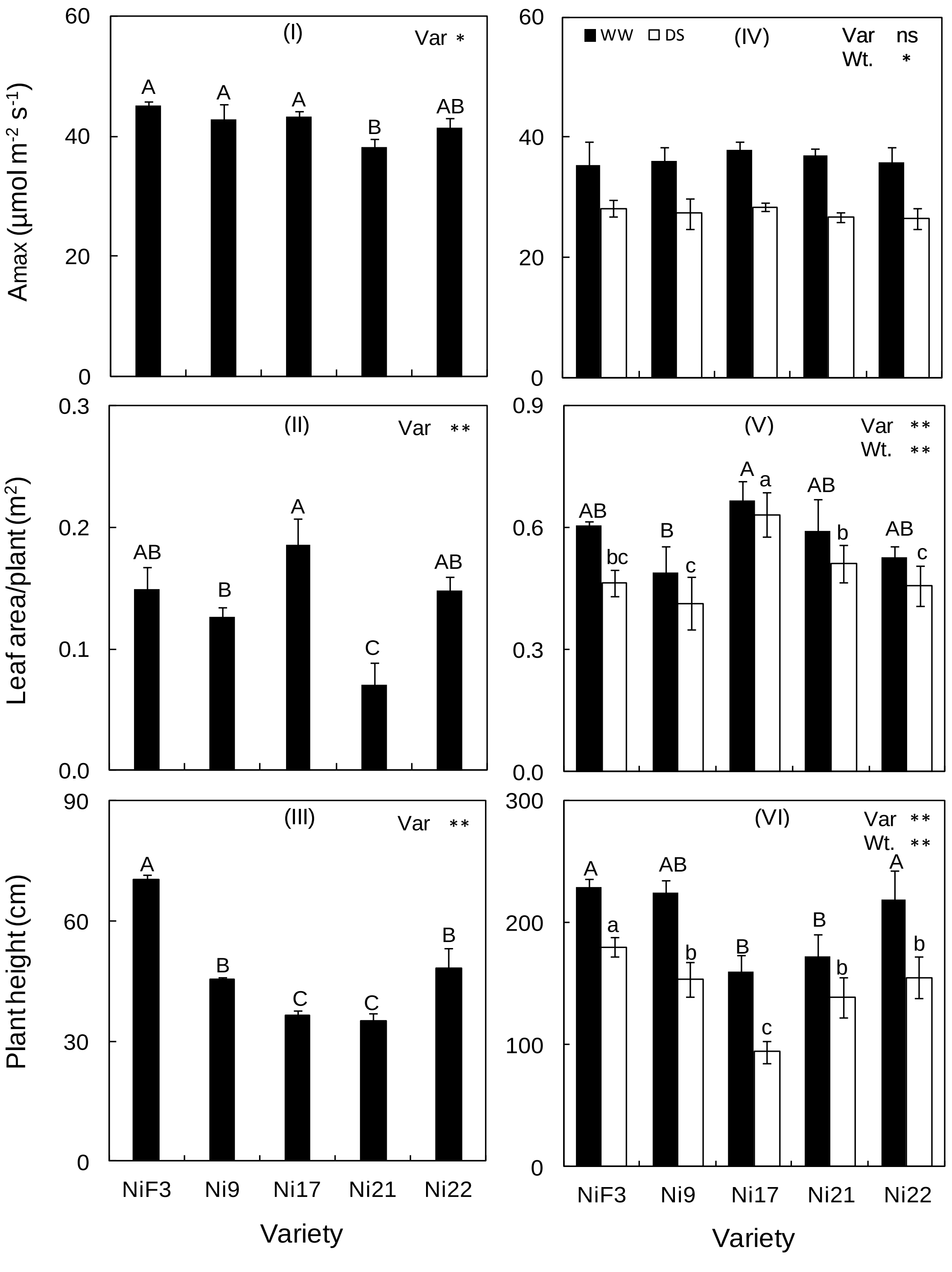

Figure 3.3. Potential photosynthetic rate (A_{max}), leaf area and plant height of sugarcane varieties under different water regimes at 60DAT (I, II, III) and 120DAT (IV, V, VI), respectively

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.

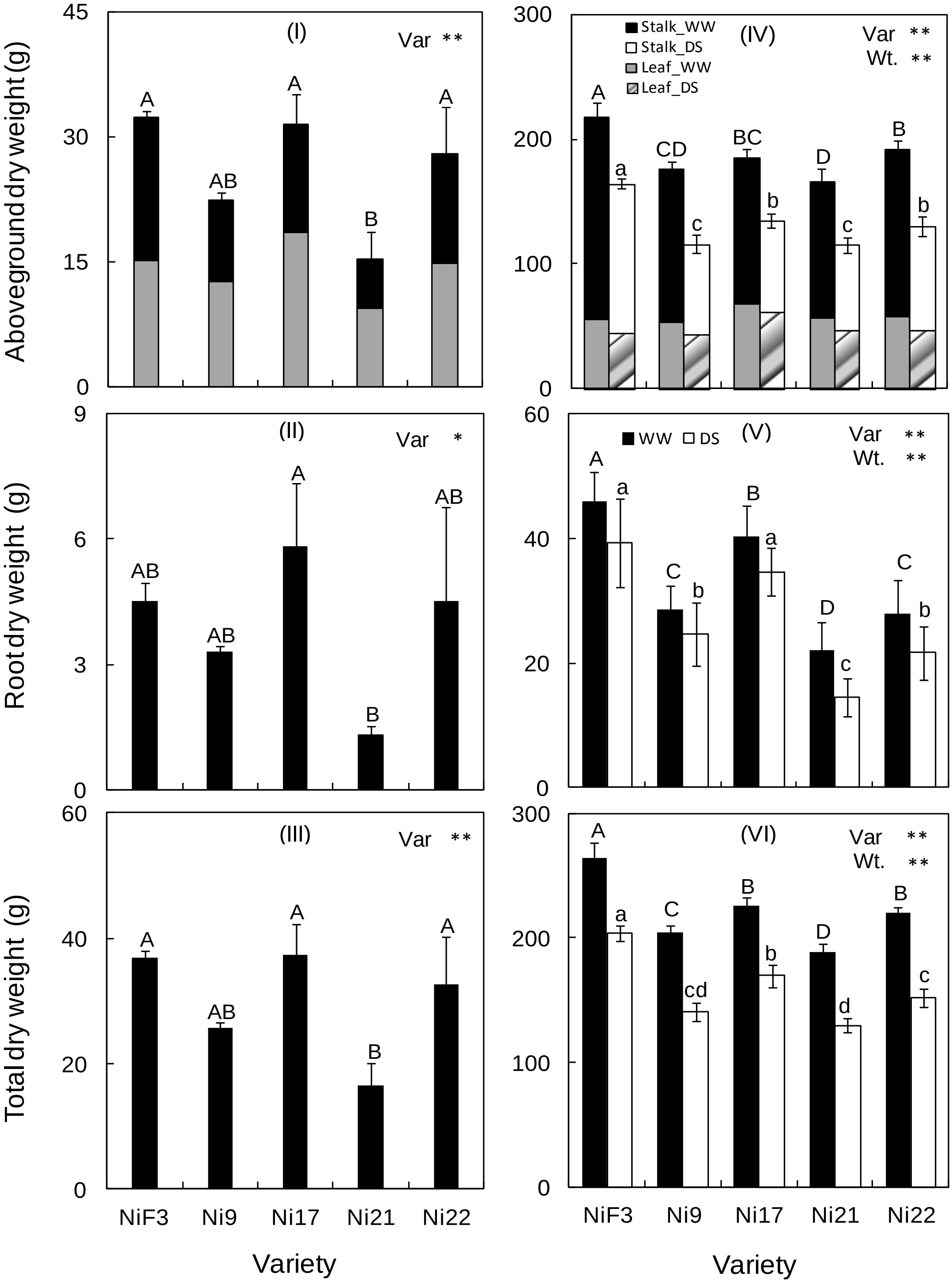

Figure 3.4. Aboveground (leaf and stalk), root and total dry weight of sugarcane varieties under different water regimes at 60DAT (I, II, III) and 120DAT (IV, V, VI), respectively

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.

From Figure 3.2, 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 both well-watered 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 well-watered condition.

The effects of different sugarcane varieties and water regimes on A_{max} and growth parameters were shown in Figure 3.3. At 60DAT, A_{max} of sugarcane varieties ranged from 38.2 to 45.2 µmol m^{-2} s^{-1}. A_{max} of Ni21 was lowest and significantly lower than those of NiF3, Ni9, and Ni17 (Figure 3.3. I). However, at 120DAT, there were no significant differences in A_{max} of varieties under both well-watered and stress conditions (Figure 3.3. IV). At this time, drought stress affected A_{max} by the reduction rates of 19.4 to 26.1%. Ni21 had the highest reduction rate, whereas NiF3 did the lowest one, but the differences were not remarkable among target varieties (data not shown). Different varieties had significant differential effects on leaf area and plant height at both 60DAT and 120DAT. In fact, at 60DAT, leaf area of Ni17 was highest and significantly higher than that of Ni9 and Ni21. Ni17 also had the highest leaf area at 120DAT and significantly higher than Ni9 did under the well-watered condition and all other varieties did under stress condition. Ni21 had the 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 3.3. II, V). At 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 both water conditions, and higher than all other varieties under drought stress condition. Meanwhile, Ni17 and Ni21 did the lowest plant height under both conditions (Figure 3.3. III, VI). Drought stress reduced significantly leaf area and plant height of all target varieties with the reduction rate of 6.9 to 21.3 % and 19.4 to 41.2%, respectively (Figure 3.3. V, VI). 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 3.4, 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 well-watered condition (Figure 3.4. IV-VI). Ni21 and Ni9 showed the lowest values for dry weights than others at 60DAT (Figure 3.4. I-III). They also did the lowest values for all traits under both water conditions at the later period (Figure 3.4. IV-VI). At 120DAT, NiF3 had the highest aboveground dry weight, root as well as total dry weight, which followed by Ni17 and Ni22 at both well-watered 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 eight were shown in NiF3 and for leaf dry weight in Ni17, respectively (Figure 3.4. IV-VI).

Drought stress significantly reduced PNUE, TNUE, and BNUE of sugarcane varieties (Table 3.2) by the reduction rates 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, TNUE, BNUE, and DTI. 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 TNUE, and BNUE under both water conditions, but TNUE 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, TNUE, and BNUE.

The interactions between water regimes and varieties were not significant for N related traits (Table 3.2), A_{max}, growth and dry matter parameters (data not shown) It indicated that variety with high potential for target traits under well-watered conditions performed well under drought stress condition at the early growth stage.

DTI had positive significant correlations with PNUE (r= 0.66**), TNUE (r= 0.58*) and BNUE (r= 0.76**) (Figure 3.5). The correlations of partial DTIs and NUEs also showed positive relationships (Figure 3.6). Ni17 had highest partial DTI and NUE calculated by leaves dry weight (DTI_leaves and NUE_leaf), whereas NiF3 showed the highest partial DTIs and NUEs calculated by the stalk (DTI_stalk and NUE_stalk) and aboveground dry weight (DTI_above and NUE_above). 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 3.3).

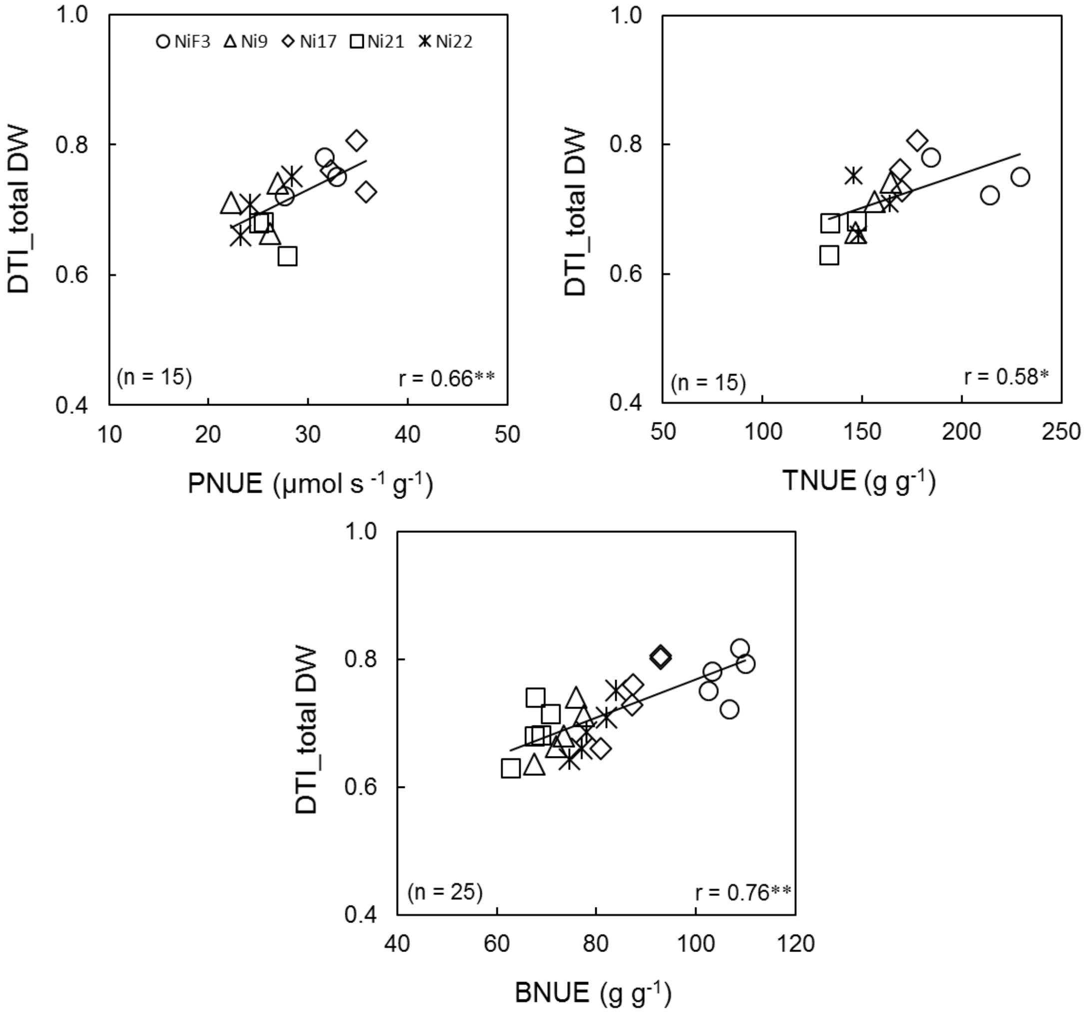

Figure 3.5. Correlations among total dry matter-based drought tolerant index (DTI_total DW) with photosynthetic nitrogen efficiency (PNUE), biomass nitrogen utilization efficiency (TNUE) and biomass nitrogen use efficiency (BNUE) of sugarcane varieties at 120DAT

* and ** mean significant at $P < 0.05$ and $P < 0.01$, respectively.

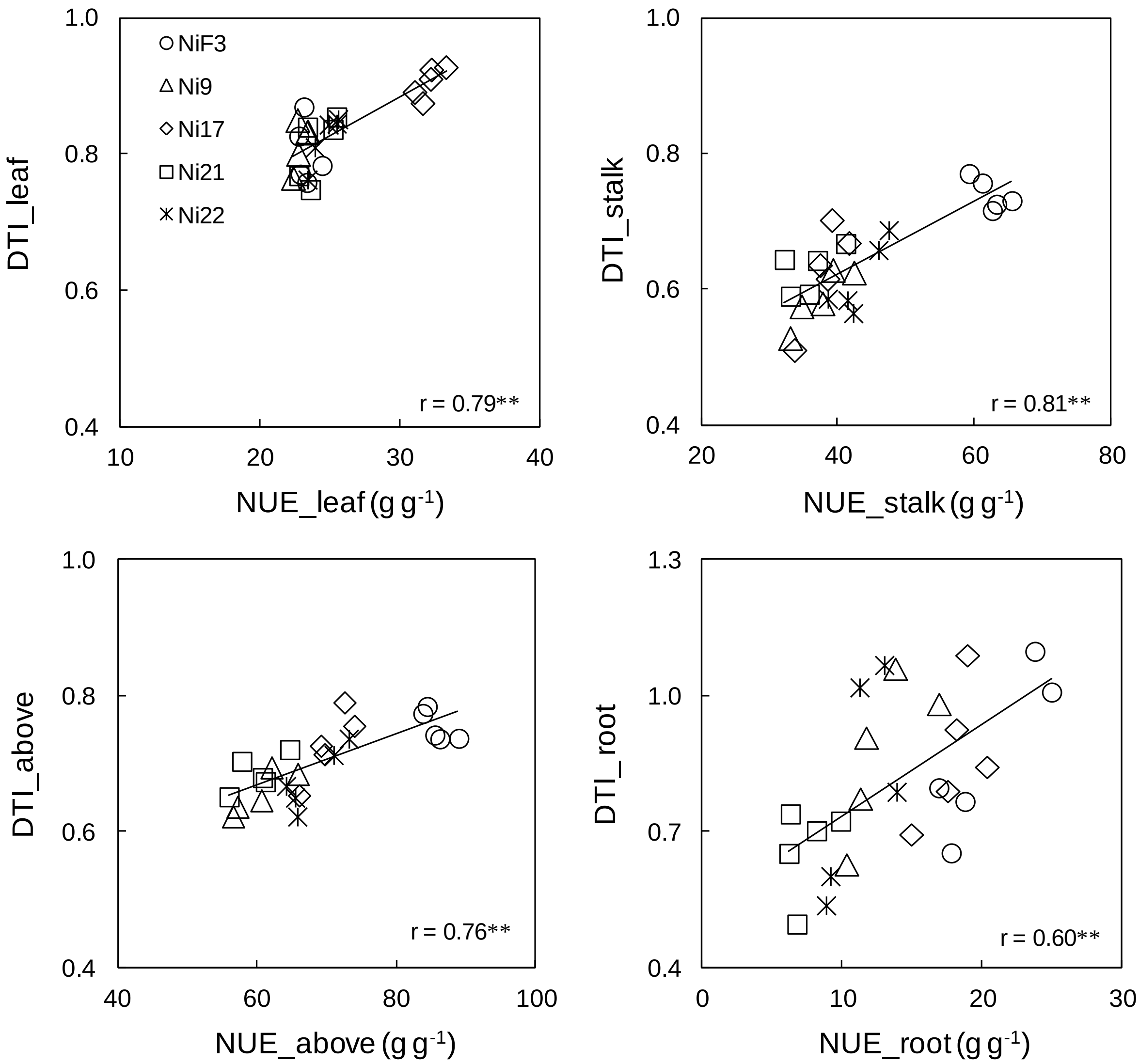

Figure 3.6 Correlations among partial drought tolerant indexes (DTI) and biomass nitrogen efficiencies (NUE) for aboveground parts (including leaves and stalk) and underground part (root) of sugarcane varieties at 120DAT

* and ** mean significant at $P < 0.05$ and $P < 0.01$, respectively. DTI_..., NUE_...- partial drought tolerant index and nitrogen use efficiency calculated by root, leaf, stalk and aboveground dry weight, respectively.

Table 3.3. Contributions of partial drought tolerant indexes to total dry-matter based drought tolerant index (DTI)

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

ns and ** mean non-significant, significant at $P < 0.01$. DTI_...- partial drought tolerant index calculated by root, leaf, stalk and aboveground part dry weight.

3.4. 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; Chapter 2.1; Graça et al., 2010; Ribeiro et al., 2013), plant height and plant elongating rate (Barbosa et al., 2015; Chapter 2.1; Ethan et al., 2016; Zhao et al., 2010), leaf number and leaf area (Barbosa et al., 2015; Chapter 2.1; Robertson et al., 1999). The decrease of source and sink (photosynthesis followed by vegetative tissue growth) leads to declining dry matter accumulations in both partial and whole plant. The results of this study 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; Chapter 2.1; Zhao et al., 2010; Robertson et al., 1999; Wagih et al., 2003).

Genetic variations in photosynthetic rate, leaf area; tops, root and total biomass were found among different sugarcane varieties (Basnayake et al., 2012; Jackson et al., 2016; Li et al., 2017; Luo et al., 2014; Ramesh, 2000). In this study, the genetic variations were also found in growth and dry matter parameters of sugarcane varieties at both 60DAT and 120DAT, whereas the difference in A_{max} was just among Ni21 with NiF3, Ni9, and Ni17 at 60DAT. At 120DAT, there were no significant differences in A_{max} under both drought stress and well-watered condition. The difference that did not find in A_{max}, but in the partial and total dry matters, hence, made the differences in photosynthetic efficiency of investigated varieties. Previous studies reported that drought-tolerant varieties have better performance as well as lower reduction rates of growth and biomass parameters in comparison with drought sensitive ones (Begum and Islam, 2012; Hemaprabha et al., 2013; Silva et al., 2007; Wagih et al., 2003). In this study, NiF3 and Ni17 presented better performance under both water conditions with the lower reduction rates 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).

In this study, drought stress increased significantly TN, but it did not affect NL (Table 3.2). The result in Chapter 2.1 showed that NL seemed to reduce at lower N application levels but increased at higher levels when plant subjected to water stress. Similarly, Ludlow et al. (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 N 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 N ability (in the form of urea which was applied just 10 days before plant subjected to water stress) which led to reducing the amount of N uptake in Silva et al. (2017)'s experiment. Meanwhile, in this study, the dissolved N 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, N 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 et al. (1998); Ranjith and Meinzer (1997); Robison et al. (2009); Robinson et al. (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, N is used to create new organs throughout assimilation and remobilization processes by reductase and synthetase enzymes i.e. nitrate reductase or glutamine synthetase (Lattanzi et al., 2015). The reduction of NUE through nitrate reductase activity when sugarcane subjected to drought stress and the NUE variation between two selected cultivars were reported (Abayomi, 2001). In this study, although the same amount of N uptake, differences in the accumulated dry matter between water regimes and among sugarcane varieties showed significant differences NUE were contributed from N utilization efficiency rather than N uptake efficiency.

The positive correlation among DTI and NUE parameters (Figure 3.5) suggested that higher NUE traits, especially higher BNUE 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 other 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 a clear different style with shorter stalk but larger leaf area than others. Trying to evaluate the contributions of partial DTIs to total DTI, I found that aboveground contributed more than root did, and stalk had a larger contribution than leaf did. From this result, it can suggest that DTI calculated by aboveground dry weight could be used as a replacement for DTI calculated by total dry weight in evaluating drought-tolerant ability. Moreover, DTI calculated by stalk dry weight should be used along with DTI calculated by aboveground/total dry weight as an extra evaluation.

In this study, NiF3 (high remained stalk weight) seemed to be better for the tolerant ability to 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 the leaf senescence (Plant Variety Protection [PVP], http://www.hinshu2.maff.go.jp) or the reduction of stalk length after drought stress period. By this evaluation, NiF3 was also considered as a strong tolerant variety (National Agriculture and Food Research Organization [NARO], 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 [OPG], 2015). However, in this study, Ni17 seemed to be better tolerant to drought stress (not significant) than Ni9, Ni21, and Ni22 (Table 3.2). It is quite difficult to compare this study to actual field evaluation for drought tolerance, because of several differences. This study 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, this study 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 the growing condition is under rain-fed conditions. For instance, in other reports, Ni9 was considered as a relative (Matsuoka, 2006) or a little weak tolerance (Alic, https://www.alic.go.jp) to drought stress. Similar to Ni17, NiF8 is often considered as a little weak (OPG, 2015) or a medium type for drought stress (PVP, http://www.hinshu2.maff.go.jp), but in NARO's report, it was also considered as a tolerant variety (NARO, http://www.gene.affrc.go.jp). 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 drought tolerant ability of investigated sugarcane varieties, the further demonstrations under non-limited conditions at the field scale should be practiced in later studies.

CHAPTER IV

LEAF PHOTOSYNTHETIC RESPONSE TO CHANGING OF SOIL MOISTURE CONTENT IN SUGARCANE

4.1. Introduction

Agriculture is the largest water consumer. Over 70% of the globally available freshwater withdrawals are used for agricultural irrigation. By estimation, the global water demand for agriculture will increase by a further 20% in the next 50 years due to irrigational needs (Global Agriculture, http://www.globalagriculture.org). However, the freshwater source using for agriculture becomes more and more shortage because of the impacts of climate change and the competition from other economic sectors. Therefore, saving and more efficient water use should be an important part of any agricultural development strategy. Recently, using the soil moisture sensor, in the smart agriculture theme, is one of the handy and simple ways to manage agricultural irrigation. It is easy to set up an automatic irrigation system by using the output data from soil moisture loggers to connect to a smart computer. However, it is essential to detect the time to start irrigation.

Sugarcane is one of the most important cash crops that accounts for 80% of global sugar production. It is also used as an important source to produce alternative fuel. Approximately 40 - 50% of the world bio-ethanol production is based on sugarcane (Zuurbier and Vooren 2008). At the different growth stages, sugarcane’s green tissues contain 60 - 80% water. Thus, in its life cycle, sugarcane requires a huge water amount with 1500-2000mm of annual rainfall to create an optimum production (Food and Agriculture Organization of the United Nations, http://www.fao.org). Hence, the water deficit that brings about reductions in photosynthesis, growth and biomass accumulation may be the reason for revenue failure. Basnayake et al. (2012) reported that water deficits by half irrigation and rainfall dependence reduce total dry matter and cane yield of sugarcane genotypes by 20 - 56% and 17 - 52%, respectively. Water supplement to compensate for water shortage from rainfall, therefore, is requisite. However, when irrigation reaching an optimum level or soil moisture attaining to an optimum content, more water applied or higher soil moisture content will not give any more advantages. Wiedenfeld and Enciso (2008) reported that there were no significant differences in cane yield and sugar yield when increasing water application from 80 to 120% of crop water requirement. Similarly, the cane and sugar yields increased significantly when water application was increased from 0.6 to 1.0 IW/CPE (irrigation water/cumulative pan evaporation), but not significantly when increasing irrigation levels up to 1.2 IW/CPE (Singh and Mohan 1994; Bahrani et al. 2009). The decision of time for irrigation to maintain high and economical yielding become importance. Basing on the change of soil moisture, Ibrahim (1978, 2006) reported that starting irrigation at 40 - 50% depletion of available soil moisture (DASM) is the best time to keep the highest yielding, but irrigation at 60 - 70% DASM giving the economic yields. Delaying irrigation to 70% DASM was detrimental to the growth of sugarcane and resulted in economic losses of sugar yield. The changes in physiological traits (such as stomatal conductance, transpiration rate, internal CO_2 concentration, and photosynthetic rate) along with morphological traits (such as stalk and leaf growth) are the most common initial adaptation when sugarcane plant subjects to mild to moderate dehydration (Inman-Bamber and Smith 2005; Ferreira et al. 2017). These physiological and morphological changings could be used as indicators to decide an irrigation schedule. In fact, leaf and stalk extension, green leaves number were suggested as indicators to decide the irrigated time to avoid reductions in biomass accumulation, with an irrigation trigger point at which stalk elongation is reduced by 50% because of water deficit (Inman-Bamber 2004). Because of photosynthesis is highly sensitive to water deficit (Ghannoum 2009), it is not only easy to reduce when soil moisture content decrease, but also easy to recover when re-watering (Chapter 2.2), in this recent study, we tried to apply the photosynthetic parameters as indicators to decide irrigation schedule. The daily changes of soil moisture and photosynthesis of sugarcane when plant subject to water deficit and after re-watering was investigated to point out the critical soil moisture value that will be helpful information for a smart irrigation system in sugarcane.

4.2. Materials and methods

Experimental design

A root-box experiment was conducted from 20 April to 16 December 2016 under glass-house conditions at the University of the Ryukyus, Okinawa, Japan (26º25'N, 127º45'E; altitude 126 m). The two root-boxes (RB-A and RB-B, 93 x 8 x 96 cm) were filled up to 90 cm of height by 2 mm sieve mixture of Shimajiri Mahji red soil: sea sand: peat moss (1: 1: 1, v v^{-1}). In each root-box, volume water content sensors (5TE soil moisture and temperature, Decagon Devices Inc., USA) were installed at 5, 25 and 50 cm of depth; and a soil matric potential sensor (MPS-6, Decagon Devices Inc., USA) was also installed at 25 cm of depth. A vertical soil temperature sensor (VTS-1, ADS), which can record data in each 1cm, was also installed from ground to 30 cm of depth (Figure 4.1).

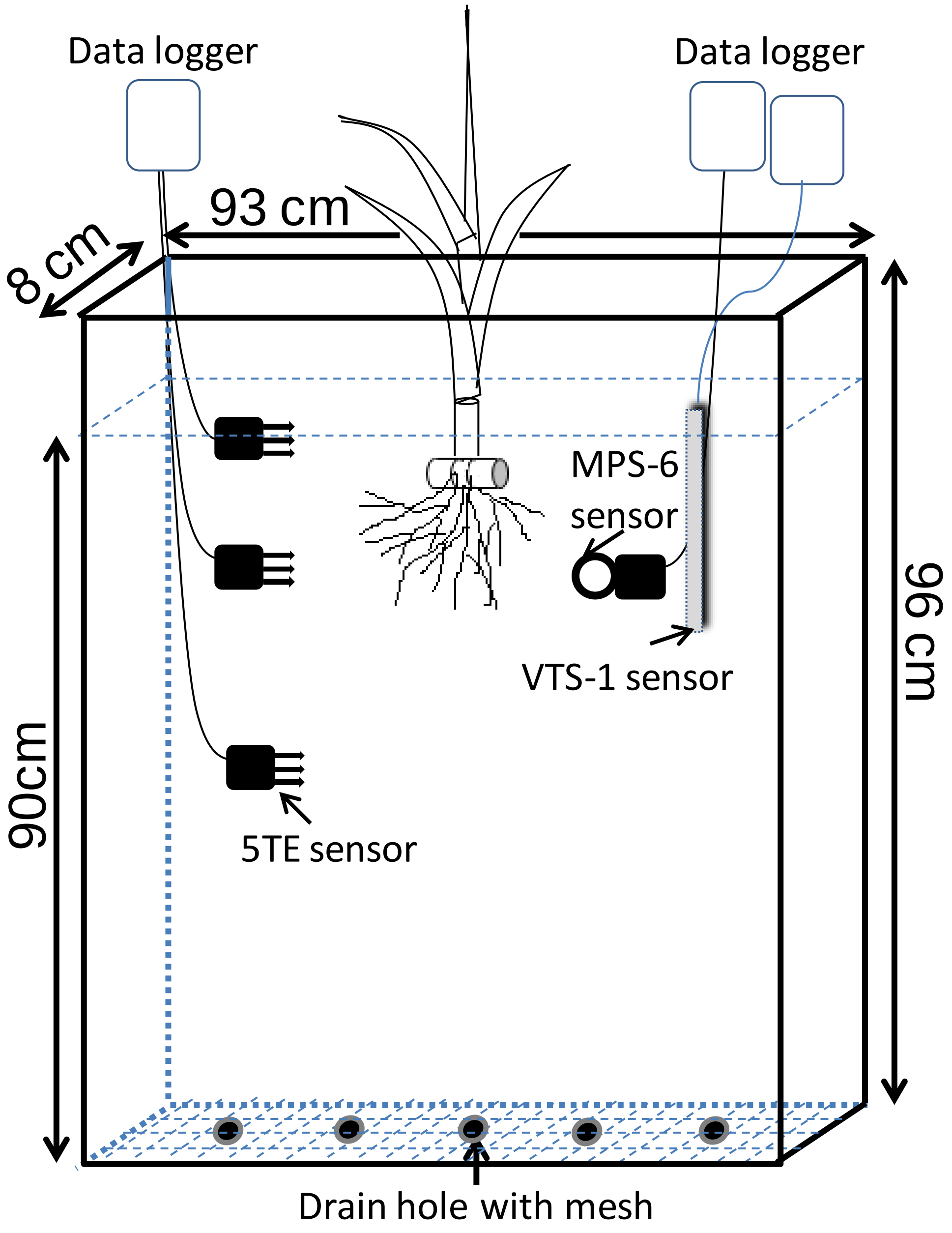

Figure 4.1. Root box design with soil sensors

The 2-month-old seedling of the commercial sugarcane variety NiF8 was transplanted, and then water was irrigated from the topsoil to fill up the box. After that, water was supplied every day to replace daily water loss. Until 4 November, water was withdrawn for 22 days with RB-A. The two days prolonging the most severe drought period to see more clearly effect of water deficit, as well as the sensitive of leaf photosynthesis to re-watering, was done with RB-

B. After the stress period, re-irrigation was done until the end of the experimental period. Plant in each root-box was fertilized weekly by replacing irrigation with 500 mL of the modified Hoagland's nutrient solution with a composition of 6mM $Ca(NO_3)_2.4H_2O$, 4mM KNO_3, 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 $C_{10}H_{12}FeN_2NaO_8.3H_2O$.

Data collection

The outdoor and indoor climatic parameters including air temperature and relative humidity, vapor pressure, solar radiation, and precipitation were recorded 10 minutes interval by weather systems (Harusa View, ADS) which were installed beside and inside the glass house. Soil physical parameters including moisture content, temperature, and electric conductivity were recorded by MPS-6, 5TE and VTS-1 sensors for each 10 minutes interval.

Photosynthetic parameters including potential photosynthetic rate (*A*), stomatal conductance (g_s), transpiration rate (*E*) and internal CO_2 concentration (C_i) were daily determined at the same first and second fully expanded leaves from 1 day before stress period until the end of experimental period by a LI-6400 portable photosynthesis system (Li-COR, Lincoln, Nebraska, USA) equipped with a 2 x 3 cm^2 LED chamber between 900 to 1500hr at a photon flux density of 2000 $\mu mol\ m^{-2}\ s^{-1}$, leaf temperature of 33 ± 2 °C and CO_2 concentration of 450 $\mu mol\ mol^{-1}$ which close to glasshouse air condition. SPAD values were also recorded as soon as photosynthesis measurement at the same positions by a SPAD meter (SPAD- 502, Minolta, Japan).

4.3. Results

Overall, the large differences in environmental parameters between indoor and outdoor conditions were shown at the midday. In fact, the indoor air temperature and relative humidity fluctuated from 16.4 to 37.3 °C and from 29.5 to 94.5 %, higher than outdoor from -2.3 to 13.7 °C and from -10.3 to 41.1 %, respectively. The solar radiation changed from 0.0 to 645.0 W m^{-2}, lower than outdoor from 0.0 to 630.9 W m^{-2}. The indoor vapor pressure fluctuated from 13.3 to 31.5 hPa. In open-air condition, precipitation was recorded from 0.0 to 1.8 mm. The solar radiation, air temperature, and vapor pressure had the trend to reach the peaks at midday and bottomed at midnight, whereas the air relative humidity had the opposite trend (Figure 4.2).

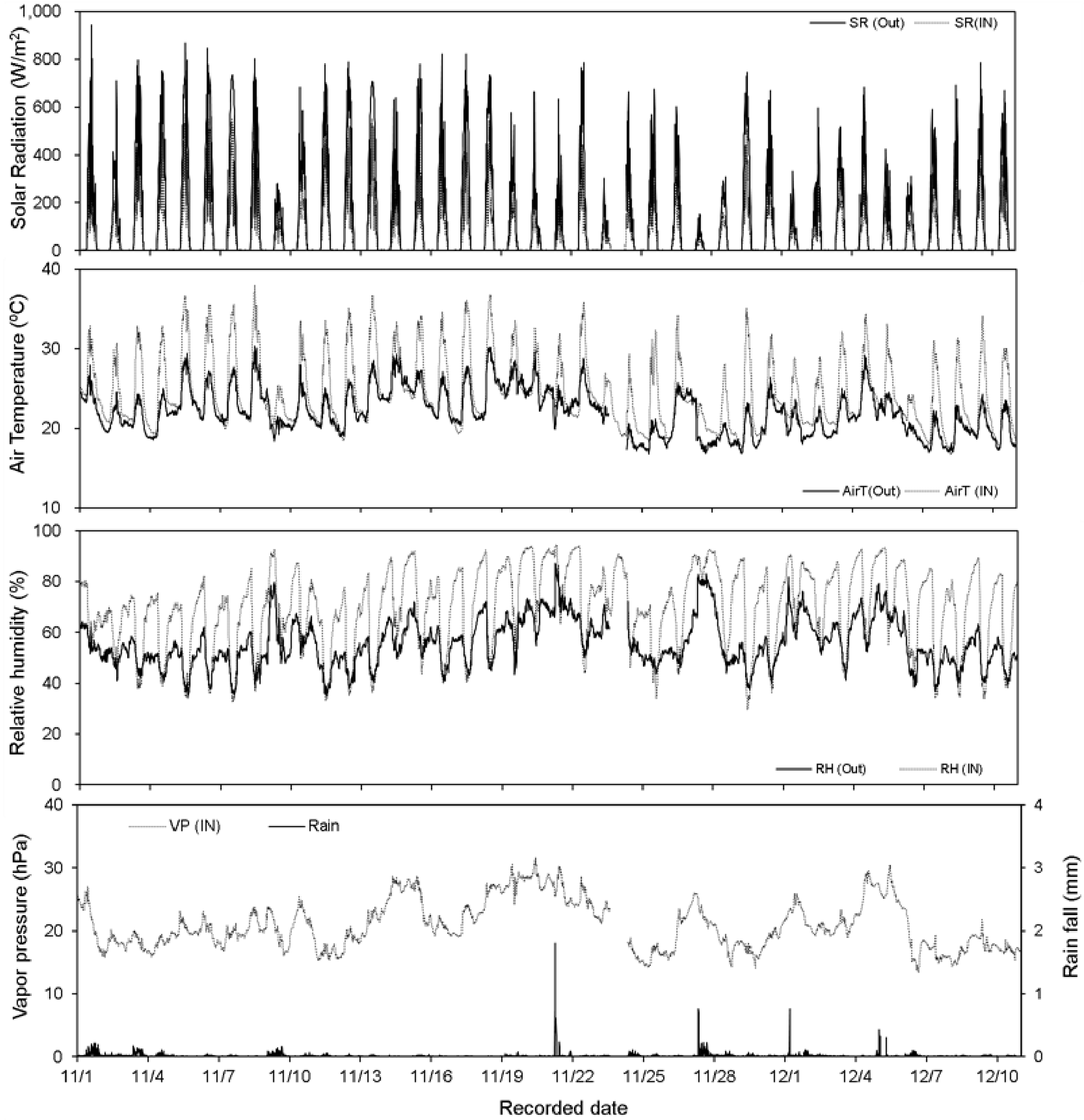

Figure 4.2. Climate data during the experimental period

SR, solar radiation; AirT, air temperature; RH, air relative humidity; VP, air vapor pressure; (Out) and (IN), weather parameters at the outdoor and indoor condition, respectively;

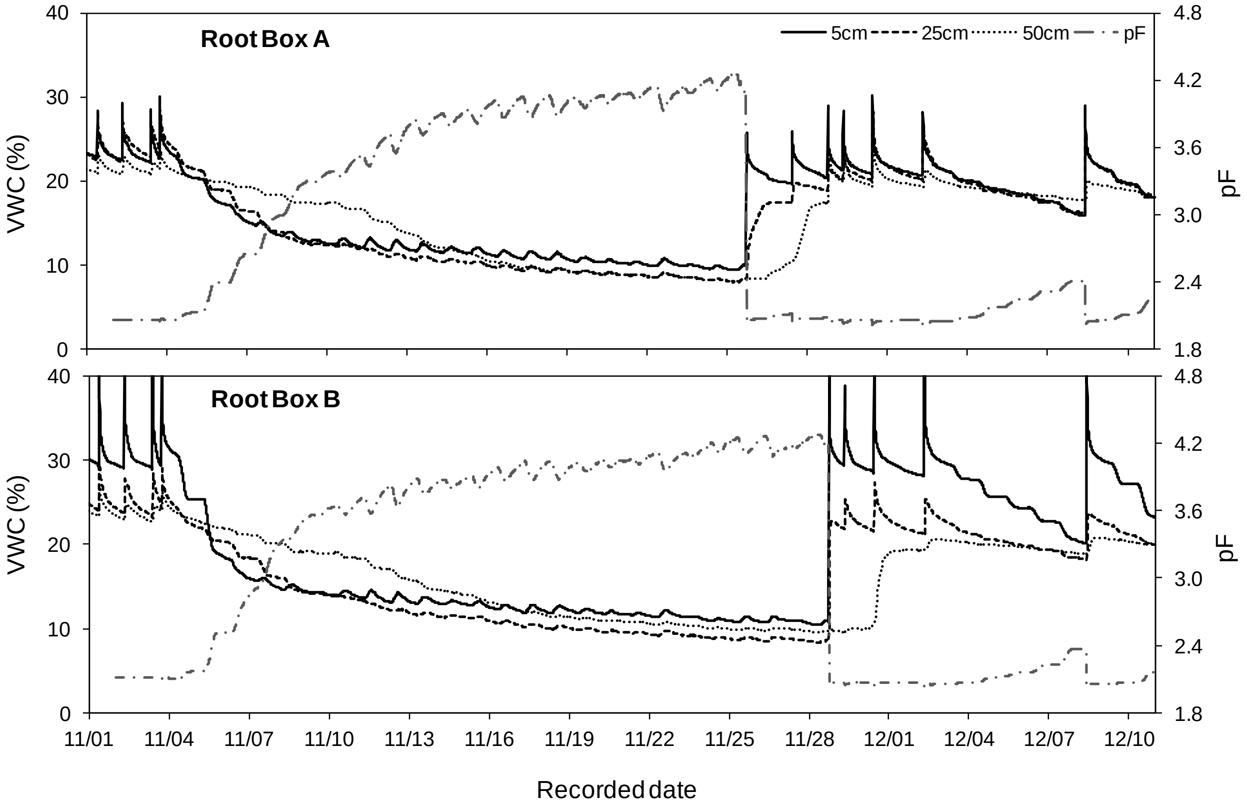

Figure 4.3. The daily change in soil moisture content during the experimental period

Air Temp and SR, air temperature and solar radiation at indoor condition; VWC, volume water content (recorded by 5TE sensors), pF, pF value (recorded by MPS-6 sensor); 5cm, 25cm and 50cm, moisture content recorded by 5TE sensors at depth of 5, 25 and 50 cm, respectively

As can be seen from Figure 4.3, the daily soil moisture increased whenever starting irrigation, then decreased during the day. It is interesting that although withholding water during a drought period, soil moisture recovered at the nighttime. Before the stress period, volume water content (VWC) which recorded by 5TE sensors ranged around 25% at three depths of 5, 25 and 50 cm in RB-A. Meanwhile, in RB-B, the same VWC values were recorded at depths of 25 and 50 cm, but at the depth of 5 cm, VWC fluctuated more widely in comparison with others. During the stress period, in general, VWC changed at the most rapid rate during the first 10 days of drought period (DDP). After that, VWC steadily declined up to 10%. The change of VWC at deeper layers were slower in comparison with other layers. During the recovery period, VWC immediately turned back to initial condition at upper layers, whereas it took 4 days at 50 cm of depth. On the contrary, pF values which recorded by MPS-6 sensor increased from 2.0 at the beginning up to 4.2 at the most severe drought stress, then rapidly dropped to non-stress value (around 2.0) as soon as re-watering. It was clearer when soil moisture values were pointed out as shown in Figure 4.4.

SPADs of the plant in RB-A fluctuated around 50 during 14 DDP before declining to 42 at 22DDP. SPAD of upper leaf, then, rapidly recovered to 45 at 3 days after re-irrigation (DAR), while SPAD of lower leaf continuously decreased to 40, and then recovered to 45 at one day later. Similarly, SPAD of the plant in RB-B declined to 40 at 24DDP, and then SPAD of upper leaf recovered 3 days sooner in comparison with that of lower leaf. Photosynthetic parameters including A, g_s, and E decreased moderately during the first 10DDP before rapidly declining to zero at 21DDP. From this day, the photosynthetic activity almost ceased. However, after re-watering photosynthesis immediately recovered. Interestingly, leaf C_i steadily decreased during the first 15DDP, then rapidly increased to reach the peak at the most severe stress levels, but it also rapidly decreased to the normal value as before stress when water was again supplied (Figure 4.5).

There were the same tendencies in the responses of sugarcane for photosynthetic parameters on the changes of VWC at three levels of soil depth, but the effect of moisture stress on photosynthesis seemed to be coming earlier at 5 cm of depth and later at 50 cm of depth in comparison with 25 cm of depth (Data not showed). In this study, therefore, we showed only the relationship of photosynthetic parameters with the change of VWC at 25 cm of depth as the representative of the photosynthetic response of sugarcane on changing of soil moisture (Figure 4.6). A of the plant in RB-A reduced moderately from over 30 µmol m^{-2} s^{-1} at pF/VWC of approximately 2.0/23% to around 27 µmol m^{-2} s^{-1}, whereas A of the plant in RB-B decreased more quickly to around 22 µmol m^{-2} s^{-1} during first 4 DDP. After that, A of these plants seemed to become stable when soil moisture dropped from 2.8/15 % to approximately 3.8/10 %, then it suddenly felt down to nearly 0.0 µmol m^{-2} s^{-1} when VWC was lower than 10% and pF was higher 3.8. There were similar trends in the relationship between the change of soil moisture with the change of photosynthetic parameters including A, g_s, and C_i until pF and VWC reached 4.0 and 10.0%, respectively. From these soil moisture values, C_i changed by an opposite way to went up from below 100 µmol mol^{-1} to close to 300 µmol mol^{-1}. After re-watering, the recovery of all investigated parameters was recorded.

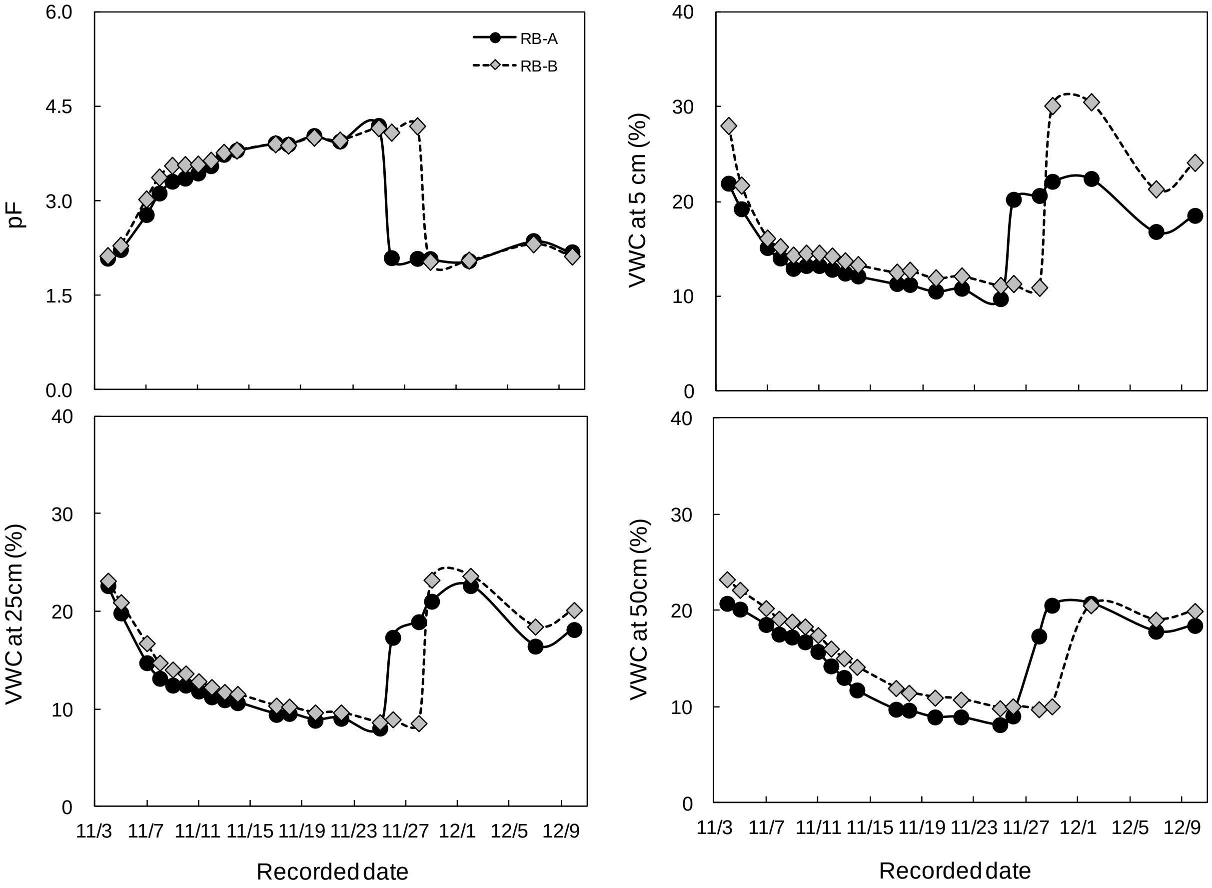

Figure 4.4. Time out of daily soil moisture during the experimental period

RB-A and RB-B, root box A and B, respectively; VWC, volume water content (recorded by 5TE sensors), pF, pF value (recorded by MPS-6 sensor); 5cm, 25cm and 50cm, moisture content recorded by 5TE sensors at depth of 5, 25 and 50 cm, respectively

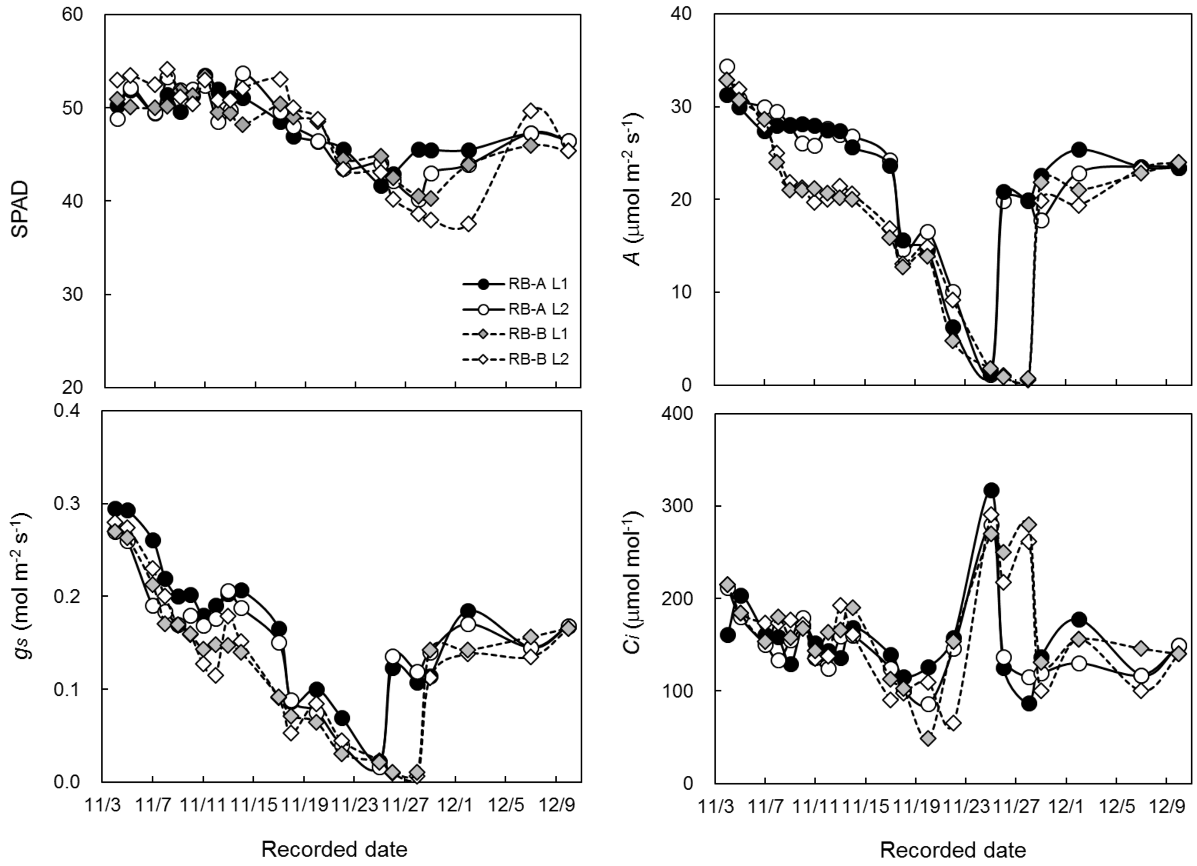

Figure 4.5. Time out of the daily change of SPAD, photosynthetic rate (*A*), stomatal conductance (g_s) and internal CO_2 concentration (C_i) of sugarcane during the experimental period

RB-A and RB-B, photosynthetic parameters from root box A and B, respectively; L1 and L2, first and second leaf, respectively;

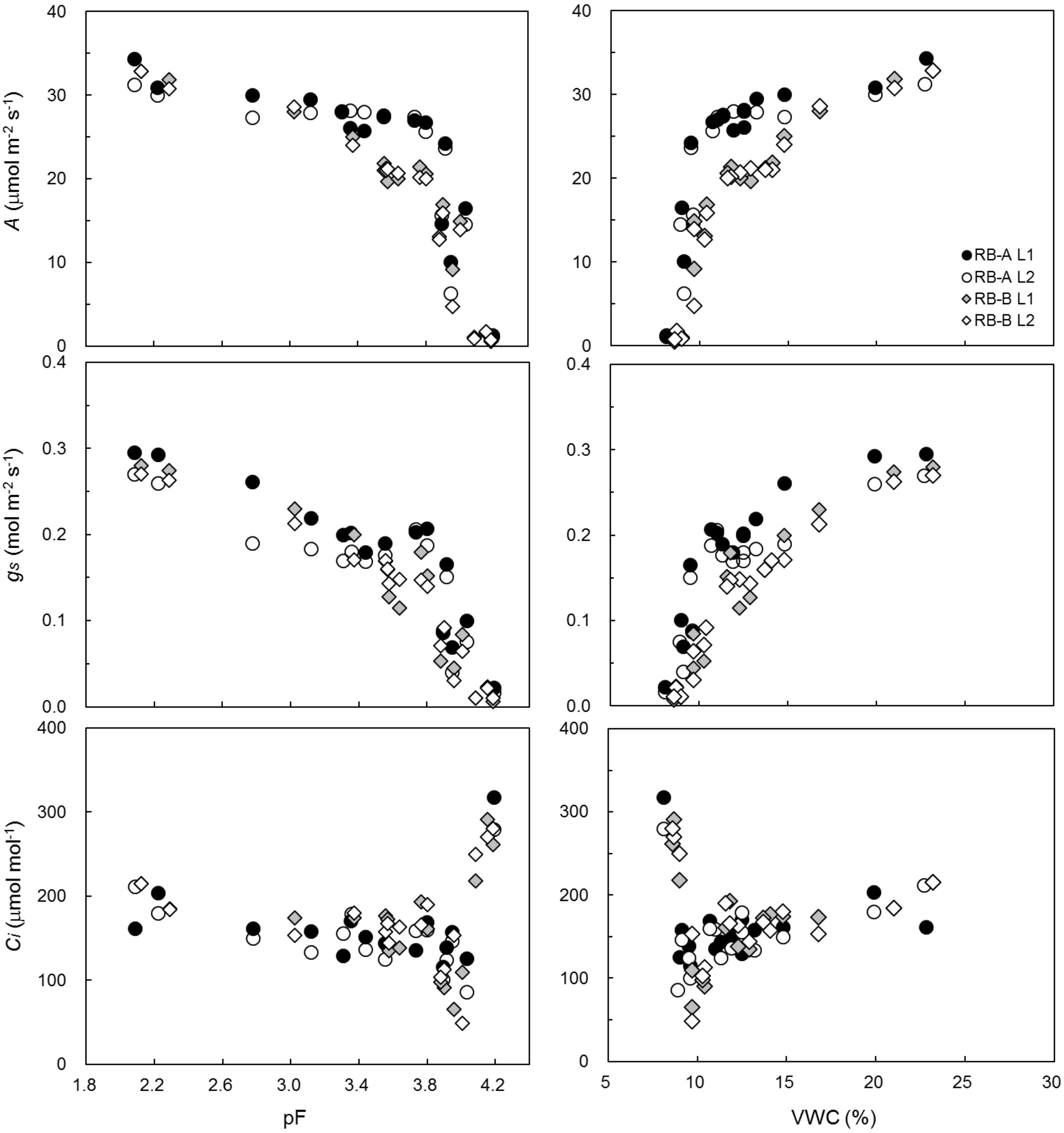

Figure 4.6. Relationships among soil moisture content (recorded by pF and 5TE at 25 cm of depth sensors) with photosynthesis rate (*A*), stomatal conductance (g_s) and internal CO_2 concentration (C_i)

RB-A and RB-B, photosynthetic parameters from root box A and B, respectively; L1 and L2, first and second leaf, respectively; VWC, volume water content (recorded by 5TE sensors) at 25 cm of depth; pF, pF value (recorded by an MPS-6 sensor);

4.4. Discussion

The air temperature, air humidity, solar radiation, wind speed, and precipitation are climatic factors that control soil evaporation and plant transpiration. In fact, reference evapotranspiration (ET) could calculate by these factors (Zotarelli et al. 2015). Moreover, solar radiation has a positive correlation with ET, and often be calculated by the daily air temperature. Increasing solar radiation and air temperature, and decreasing air humidity lead to increase vapor pressure that stimulates photosynthetic activity following by higher water loss in the plant. Therefore, in the sunny day, soil moisture decreases rapidly. Meanwhile, in the cloudy or rainy day, photosynthetic rate and transpiration rate are lower, and in certain soil moisture decrease more slowly than that on sunny days. Soil moisture at the upper layer from 0 to 25 cm of depth reduced faster than that at the lower layer (50 cm of depth) (Figure 4.3 and 4.4). It is because of that the secondary roots of sugarcane, which often concentrates in soil surface zone, at first suck dry water from subsurface layer. This leads to water shortage and stimulating the role of lower roots to find out water from deeper soil layer to help the plant to escape temporarily from drought stress.

Interestingly, during drought stress period, soil moisture had a tendency to recover at the nighttime. This was clearer in the recovery of soil moisture at depths of 5 and 25 cm (Figure 4.3). It could be explained that at the nighttime conditions (no sunlight, low air temperature, and high air humidity), the plant stops photosynthetic activities which lead to ceasing water loss from leaf transpiration as well as from the soil. Moreover, water always moves in the direction of decreasing potential from higher potential energy region (deeper soil layer) to lower potential region (upper layer) (Davidson 1989). These reasons led to the increase of soil moisture at upper soil. However, the osmotic water from deeper layer was not enough to compensate for a large water loss that is absorbed by the root system to support leaf photosynthesis in the daytime, hence, overall soil moisture still declined. Furthermore, in terms of “hydraulic lift”, Richards and Caldwell (1987) described the upward movement of water from deep wet to shallow dry soil layer. This could also explain the recovery of soil moisture at the upper layers. During the daytime, plant transpiration forces water inflow from the soil through the stem and out to atmosphere via opened stomata. At night, the close of stomata suppress transpiration which leads to equilibration between plant water potential with that of the soil where most active roots are found, which results in water potential gradients between the plant and the drier soil points; hence, water moves from roots to these dry soil layers (Kramer and Boyer 1993; Prieto et al. 2011). The hydraulic lift could be one of the mechanisms that help the plant living in waterless environments to mitigate the harmful effects of water deficit.

The result showed that there were quick reductions in *A* and g_s during the first 4DDP when soil moisture dropped in moderate deficit threshold (pF from 2.0 to 2.8 and VWC from

23 to 15% respectively). After that, they became stable during 6 days later (Figure 4.5 and 4.6). Meanwhile, relative chlorophyll content (SPAD values) maintained during the first 14DDP. It could be explained that during the first 3DDP, leaf photosynthesis of the sugarcane plant was sensitive to drought stress. Stomata closed very fast to restrict water loss and interrupted CO_2 exchange that was a reason for the decrease of *A* and C_i. In the next days, when the plant can adapt to water deficit, the water from root system at deeper layer along with water stored in the stalk supplied to maintain the amount of water transport to leaves to support photosynthesis, g_s following by *A* were maintained. However, since 10DDP, soil moisture decreased to a more severe deficit threshold of pF from 3.8 to 4.2 and VWC from 10 to 8%, which could reach the soil permanent wilting point. At this time, the senescence of lower leaves was observed, chlorophyll content in top leaves was reduced, stomata closed more rapidly leading to the reduction of *A* close to zero and plant growth seemed to be stopped. Likewise, Rodrigues et al. (2009) observed the very low *A* at moderate (8 days) and severe (10 days) stress when sugarcane subjected to dehydration condition. Under a rainout shelter conditions, Koonjah et al. (2006) found difference in *A* between the well-watered and water-stressed when the leaf water potential of the latter reached to -0.7 MPa at 15 days of water stress and reached to the lowest level of 2.2 µmol m^{-2} s^{-1} at leaf water potential of -1.6 MPa at 25 days of water stress. Zhao et al. (2013) reported that SPAD did not significantly differ between the well-watered and water stress plants when soil moisture slightly decreased during the first 10 to 15 days after initiation of the water stress treatment, but later when plants were already exposed to severe water stress, SPAD of water stress plants sharply declined. Similarly but sooner, *A* and g_s of stressed plants declined sharply and significantly lower than that of the well-watered plants from 7 to 10 days after initiation of the water stress period. It confirmed that because of leaf chlorophyll content is less sensitive and more stable during the first drought period than *A* and g_s. In the small size pot experiments by withdrawing steadily 50% of full daily water loss (Chapter 2, Chapter 3), I found that sugarcane could maintain growth during first 2 weeks, but later, when soil moisture reached to severe stress level, plant growth was stopped with very low increasing rate of plant height and total leaves number in drought stress treatments. Meanwhile, in the smaller pot, because of severe stress came earlier, Graça et al. (2010) found a decrease of photosynthesis after just 5 days of water deficit initiation. Although pot size or root zone size causes a difference in the rate to subject to severe drought stress, we could confirm that photosynthetic

parameters are susceptible to the change of soil moisture, it should be used as an indicator to detect time for starting irrigation.

It is interesting that after sharply decreasing from 200 to below 100 µmol mol^{-1}, C_i suddenly increased close to 300 µmol mol^{-1} when soil moisture still continuously reduced to VWC below 10% and pF above 4.0. This agreed with Du et al. (1996) that C_i was decreased when leaf water potential decrease from -0.37 MPa to -0.85 MPa, but below -0.85 MPa, with further decrease of leaf water potential, C_i increased rapidly. Plant maintains opening stomata to uptake CO_2, then CO_2 is translocated and fixed by photosynthetic enzymes such as ribulose-1,5-bisphosphate carboxylase/oxygenase, phosphoenolpyruvate carboxylase, NADP malic enzyme, etc. (Zingaretti et al. 2012). Water deficit caused stomatal closure as the result decreasing CO_2 uptake into leaf tissue. However, the reduction of photosynthetic enzymes under the effects of water stress (Du et al. 1996; Parry et al. 2002; Barbosa et al. 2015) led to declining CO_2 translocation and fixation. Along with stomatal closure, it caused the stagnation and increase of CO_2 concentration in leaf tissue. We consider that because of severe stress, almost at the permanent wilting point, plant expresses the disorders in physiological function, namely in the photosynthetic apparatus. If continuing to prolong this status, it will lead to plant death or stopping growth. A water supplement has to be done to rescue plant. Nevertheless, there is an argument that the increase of C_i could be from error calculation of C_i when stomata close. C_i is routinely calculated from the outward diffusive behavior of water vapor (Caemmerer and Farquhar 1981; Boyer and Kawamitsu 2011). This calculation seems to be reasonably accurate just in case of opened stomata because the effect of gas exchange through the cuticle is minor in comparison with gas exchange via stomata (Boyer et al. 1997). When stomata close, the calculation becomes more affected because of increasing the error from cuticle effects (Tominaga and Kawamitsu 2015). Hence, a direct measurement of C_i to confirm our result as well as to clear the change of C_i when stomata close under the effect of moisture stress should be conducted in further study.

Our result confirmed that photosynthetic parameters recovered as soon as re-watering. Previous studies found the recovery of photosynthetic parameters such as A, chlorophyll content or SPAD, etc. with the values equal or even higher than that in the non-stress condition or before water stress (Radha et al. 2015; Chapter 2.2). However, in our finding, those parameters could recover but not as before stress. It may because the previous studies just focus on the first or second leaf, whereas in our study photosynthesis was measured at the same leaves (these first leaves became third and fourth leaf at the later period). Leaf nitrogen which positively correlates with SPAD as well as *A*, depends on the leaf age (Allison et al. 1997). Moreover, water and nutrient rather than nitrogen are priority support younger leaves. Therefore, upper or younger leaves often recover sooner and have higher photosynthetic ability than lower or elder leaves. The earlier and stronger recovery for SPAD and photosynthetic parameters in third leaves than those in fourth leaves in this study could demonstrate the effect of leaf age to the recovery of SPAD and leaf *A* (Figure 4.5). Similarly, measuring on the same leaves, Pedrozo et al. (2015) found the reduction of leaf photosynthesis after 20 days. They also found the recovery of *A*, g_s, *E* and SPAD from water deficit that was equivalent to those in well-watered condition.

In conclusion, sugarcane photosynthetic parameters changed in parallel with the change in soil moisture, excepting for C_i that increased when soil moisture reached the most severe levels. Soil moisture decreasing to severe level led to disorder and standstill in photosynthesis. However, photosynthesis recovered in company with soil moisture's recovery. Therefore, photosynthetic parameters could be the important indicators to evaluate drought stress effect as well as to determine the time to re-irrigate to help plant maintain normal growth. From this study scale, we inform that VWC of 15% (recorded by the 5TE sensor) or pF of 2.8 (recorded by the MPS-6 sensor) should be the initial time to start irrigation to keep acceptable photosynthesis. Moreover, it should not start to irrigate later than 10% and 3.8, respectively to avoid any disorder may happen in photosynthetic activity. The research was conducted under root-box conditions where the restricted root zone may be the scope of this study. Furthermore, the response of leaf photosynthesis on soil moisture changing may be different from sugarcane varieties; and the changing of soil moisture could be different from soil types. Further studies under field condition with various sugarcane varieties and soil type should be done to get a firm suggestion before applying to the actual farmer field conditions.

CHAPTER V

SCREENING NITROGEN USE EFFICIENCY OF SUGARCANE VARIETIES UNDER DROUGHT STRESS AT EARLY GROWTH STAGE

5.1. Introduction

Sugarcane produces an enormous biomass production with an average of annually approximately 40 tons ha^{-1} (Waclawovsky et al., 2010). In its life cycle, sugarcane requires a huge amount of N and water for growth and development. The sugarcane productivity is mostly contributed by the stalk weight and stalk population. Drought stress at tillering and elongating phase (early growth stage) which reduces plant growth expressing in stunting, restriction of tillering, leads to vacant in plant population and low millable stalk, will result in yield loss of both cane and sugar yield. Therefore, drought stress at early growth stage becomes one of the most important limiting factors on sugarcane production.

In most cases, N is often applied during early growth stage to supply essential materials to critical processes such as photosynthesis, plant growth, expansion of green leaves, and tiller or sucker production. During early growth stage, N is easily lost from soil by leaching and by the process of volatilization. N deficiency reduces the photosynthetic capacity, induces stunted growth, reduces stooling and leads to low productivity (Schroeder, et al., 2014), and thereby causes the low NUE. Water stress during this stage induces low tissue water status and interferes water absorption followed by the reduction of nutrient uptake (Lopes et al., 2011) and restricting enzyme activity in the assimilation and remobilization processes (Abayomi, 2001), hence, becomes one of the reasons for N deficiency and low NUE.

Previous studies found that improvements in plant growth and biomass production in sugarcane genotypes incorporate with NUE traits (Calif and Edgecombe, 2015). Moreover, sugarcane plant with higher NUE could have better drought tolerant ability (Ranjith and Meizer, 1997; Chapter 2 and Chapter 3). Therefore, breeding for higher NUE, especially under drought stress at early growth stage is more and more important. The objectives of this study were to support the information in growth, biomass performance and NUE of sugarcane varieties, and to get a better understanding about the relationship between NUE and biomass production under drought stress at early growth stage.

5.2. Materials and methods

The experiment was conducted at the experimental field, faculty of agriculture, the University of the Ryukyus, Okinawa, Japan (26°25’ N, 127°45’ E; altitude 126 m) from April to October 2018. Experimental field was prepared out by plowing and harrowing one month before planting. The experimental soil is Shimajiri Mahji red soil type with pH=6.2 and total N content = 0.11%. Twelve sugarcane varieties including NiF3, NiF8, Ni9, Ni12, Ni15, Ni17, Ni21, Ni22, Ni25, Ni27, Ni28, and Ni29 were used as experimental materials. The two-month-old seedlings were transplanted on 19 April 2018 with a distance of 120 x 30 cm in row and plant interval, respectively. The surface drip tape (Adrilite, Adritec Group) with a distance of 30 cm between emitters, connected with an irrigation controller (Aqua Pro, Netafim Irrigation Equipment and Drip Systems, Israel) was installed in each sugarcane row to supply water immediately after transplanting. The irrigation pressure was kept around 0.2 bar to control the water flow rate at approximately 1.5L hr^{-1}. Fertilizer was supplied 3 times at a ratio of 2: 2: 2.5 by 130 kg $10a^{-1}$ of NPK16:6:6 at 7, 30 and 60DAT, respectively. During the experiment period, hand weeding was carried out to protect from the nutritional competition of weed. Pests and diseases were frequently observed and controlled when occurring. Fipronil ((±)-5-amino-1-(2,6-dichloro-a,a,a-trifluoro-p-tolyl)-4-trifluomethylsulfinylpryrazole-3-carbonitrile) 0.5% at the rate of 6.5 kg $10a^{-1}$, and Chlorantraniliprole (3-bromo-N-(4-chloro-2-methyl-6-([methylamino]carbonyl)phenyl)-1-[3-chloro-2-pyridinyl]-1H-pyrazole-5-carboxamide) 10% w v^{-1}, water solution concentration) with a 1:5000 dilution ratio at the rate of 100 L $10a^{-1}$ were applied as insecticides to control stem borers. Other crop management was done following the cultivation manual for sugarcane (Okinawa Prefectural Government, http://www.pref.okinawa.jp).

The experiment was divided into two blocks: water stress at early growth stage (under rain-fed condition during early growth stage) and control block (full irrigation). In each block, the same experimental design by a randomized complete block design with three replications was used. For control treatment, soil moisture was maintained around field capacity at soil moisture potential (pF value) of approximately 2.0 throughout the crop season. Total amount of irrigation water, applied for each plot, was calculated by crop water requirement (ET_{crop}) which was calculated following the methods described by Doorenbos and Pruitt (1992) using the crop coefficient for Naha region suggested by Hossain et al. (2005) and the reference evapotranspiration calculated according to Penman-Monteith equation (FAO, http://www.fao.org). For moisture stress treatment, water was withheld for 90 days from 70DAT.

Data collection

The climatic parameters including relative humidity (%), rainfall (mm), air temperature (ºC), solar radiation (W m^{-2}) and wind speed (m s^{-1}) were recorded daily from transplanting until the end of experimental period by a weather system (Harusa View, ADS) located close to the experimental field. Soil matric potential value (pF) was recorded 10 minutes interval by a soil matric potential sensor (MPS-6, Decagon Devices Inc., USA) which was installed from 65DAT between the third and fourth plants in each sugarcane row at the depth of 25cm.

From 30DAT, plant height was measured from soil surface to the top visual dewlap of mother stalk for 10 days interval. At the same time, SPAD was measured at the first fully expanded leaves of sample plant of each treatment in all replications using a SPAD meter (SPAD-502, Minolta, Japan).

At 160DAT, because of no difference in plant growth of sugarcane plant between water stress and control treatment (Figure 5.2), only sample plants from water stress block were taken to compare agronomical parameters among sugarcane varieties. The aboveground parts of the sample plant were cut, and the whole plant leaf blades were separated to determine total plant leaf fresh weight. Then, the leaf sample of around 250 g of fresh weight was taken to measure leaf area using leaf area meter (LI-3100, LI-COR, Lincoln, Nebraska, USA). Total leaf area was converted by the sample leaf area from the ratio of fresh weights of the sample and total plant leaf. After removing leaf blades, the total stalk fresh weight, the number of stalks, stalk height and stalk diameter were determined. The stalk sample of around 1 kg of fresh weight was taken to shred by a cutter grinder (S392, Jeffco, Jeffress Engineering, Australia). Approximate 500 g of the shredded sample was taken to determine the fresh weight and then squeezed by a hydraulic press machine to determine bagasse fresh weight. Bagasse and leaf samples, then, were dried at 80°C for 48hr to determine to dry weights. After that, total plant leaf and stalk dry weight were converted from leaf sample dry weight by the ratio of fresh weights of the sample and total plant leaf, and from sample bagasse dry weight by the ratio of ground sample fresh weight and total stalk fresh weight, respectively. Total plant dry weight was calculated by the sum of total leaf and stalk dry weight.

To determine total N uptake, the dry leaf and bagasse samples were separately ground by a power grinder (MN-02C Master T-429, Taiwan). Then, 25 mg of sample was taken to determine N concentration using an N/C analyzer (NC-90A, Shimadzu, Japan). Total N uptake (TNU) was calculated by total leaf and stalk N content. Then, nitrogen utilization efficiency (TNUE) was calculated by the following formulas:

TNUE (g g^{-1}) = total dry weight/ total N uptake;

Data analysis

The data were subjected to analysis of variance according to a randomized complete block design using Statistix 8.0 package. Least significant difference (LSD) test was used to compare the means.

5.3. Results

Meteorological conditions in the experimental site were shown in Figure 5.1. During the experimental time, the daily average air humidity and solar radiation in ranged from 51.9 to 92.6% and from 16.3 to 337.2 W m^{-2}, respectively (Figure 5.1a). The daily average air temperature increased from 19.1 to 28.6°C. Wind speed fluctuated from 0.6 to 5.0 m s^{-1}. Total precipitation during the experimental period was 734.0mm, mainly from early June to mid-September with a small typhoon in early July (Figure 5.1b). This precipitation condition may disturb water stress duration in this study.

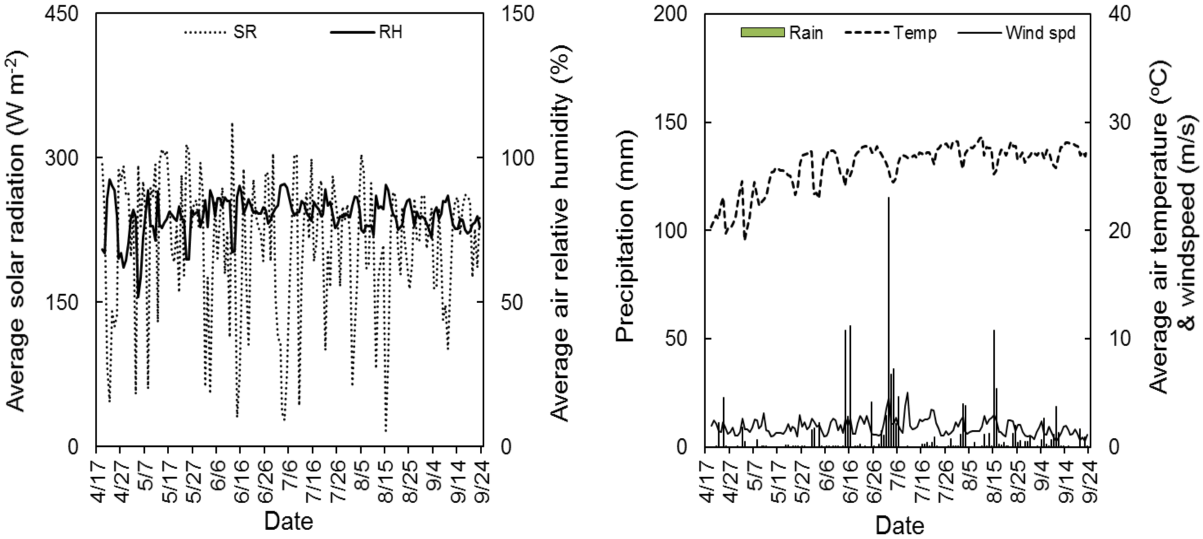

Figure 5.1. Weather conditions during the experimental period

As can be seen from Figure 5.2a, soil moisture content (soil matric potential – pF value) of control treatment fluctuated around 2.0 during the experimental period. In the water stress block, although the withholding water was conducted from 70DAT (28 June), under the effect of rainfall, pF maintained around 2.0 until 80DAT (9 July). After that, it increased to approximately 3.3 at 93DAT, then decreased to about 2.5 by a light rain at 95DAT, and increased again to the peak of 3.8 on 2 August (105DAT) before dropping to 2.1 with the effect of rainfall from 2 to 3 of August. Subsequently, pF increased with some peaks (which never over 2.8), but immediately dropping to field capacity values because of disturbance from short rainfall periods.

During the first 2 months after transplanting, the average plant height increasing the rate of sugarcane varieties was slow with around 1.0 cm day^{-1} in both water treatment blocks (Figure 5.2b). It, then, rapidly increased with the highest rate of about 3.5 cm day^{-1} during the period from 63 (21 June) to 72DAT (30 June). This increasing rate might be affected by the fertilizer application at 60DAT. Afterward, the increasing rate in the control block was reduced and maintained stably around 2 cm day^{-1}. In the water stress block, the increasing rate was lower than that was in the control block during the period from 81 (9 July) to 102DAT (30 July). The clearest difference was found during the most stressful period when pF reaching the highest values from 92 to 102DAT. Subsequently, it might be affected by rainfall, the plant height increasing rate of sugarcane plant in the stress block recovered and maintained at the same rate as that in the control block. The water stress during the short period from 80 to 105DAT might have a certain effect on the increasing rate of average plant height of sugarcane varieties, which resulted in lower of plant height in water stress treatment in comparison with that in control treatment (Figure 5.2c). However, because of the appearance of rainfall alternated with water stress period, the difference in plant height between two water regime treatments was not significant.

From Figure 5.2d, there was a downward trend of average SPAD of sugarcane varieties from approximately 52 at the beginning (17 May) to around 46 at 63DAT (21 June). Then SPAD maintained at the same levels during 20 days before declining to around 42 on 20 July (92DAT). The difference between SPAD of two water treatment blocks was found clearer, but not significant, from this date with lowers SPAD values of water stress treatment compared to those of control treatment. Since 123DAT (20 August), SPAD of water stress treatment became similar to that of control treatment.

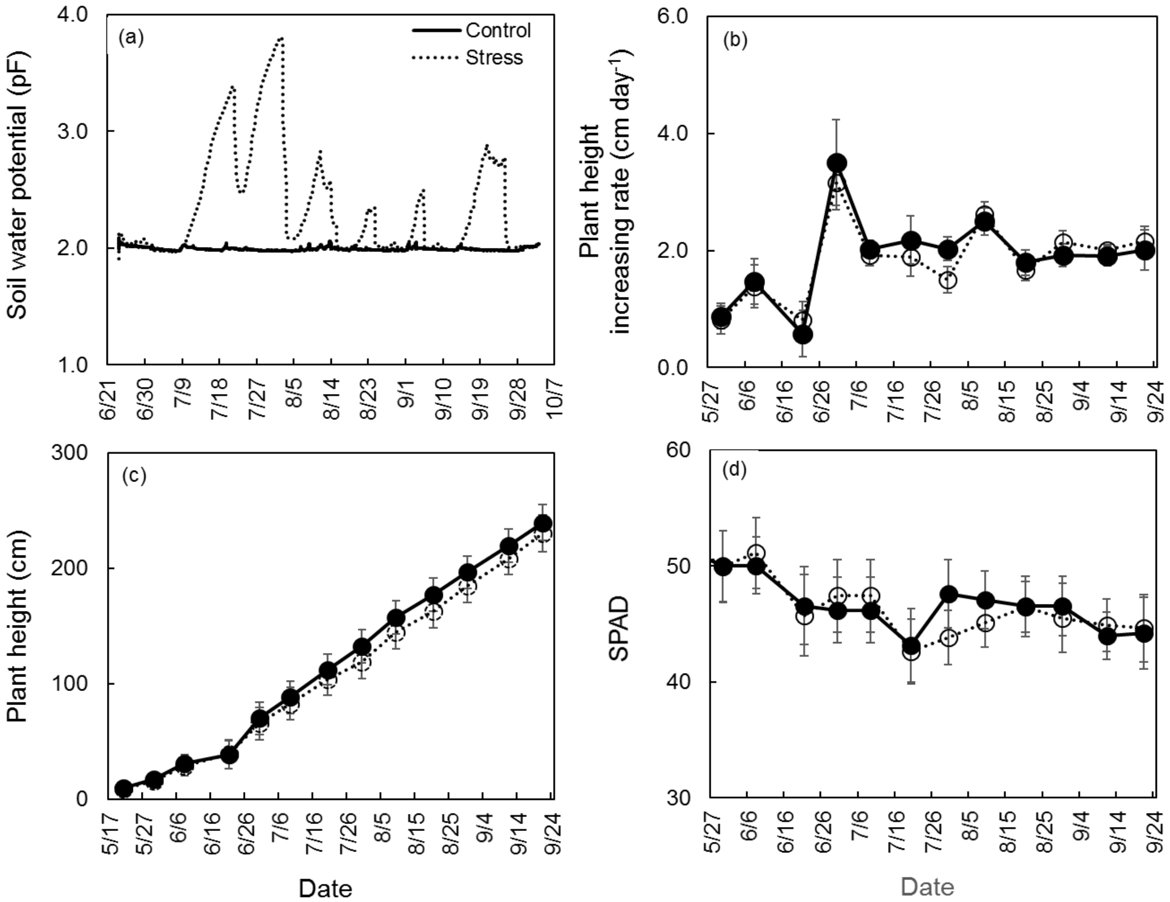

Figure 5.2. Soil water potential and growth of plant height and SPAD under different water regimes

Several agronomical parameters of sugarcane varieties were shown in Table 5.1. Sugarcane varieties tillered concentrative from 26 to 42DAT. Ni27 was the earliest tillering variety followed by Ni29 with 26 and 28DAT, respectively. Ni21 was the latest tillering variety, later than other varieties about 7 to 16 days. There were significant differences among sugarcane varieties in leaf area, tiller number, average stalk length, stalk fresh weight, and stalk perimeter. Actually, leaf area of sugarcane varieties ranged from 1.40 to 2.53 m^2. NiF8 had the largest leaf area, significantly higher than Ni12, Ni15, Ni21, Ni25, and Ni29. Tiller number ranged from 3.7 to 8.0 tillers plant^{-1}. NiF8 had the highest tiller number, whereas Ni21 showed the lowest one, but significantly lower than NiF8 and Ni9 did only. Stalk height of sugarcane varieties ranged from 216.7 to 260.5 cm. NiF3 showed the highest value and significantly higher than other varieties did, excepting for NiF8, Ni22, Ni27, and Ni28. Meanwhile, Ni15 had lowest stalk height and significantly, which were lower than those of five above varieties were. NiF3 also showed the highest values for stalk fresh weight (1.23 kg stalk^{-1}) and stalk diameter (2.6 cm), significantly higher than all other varieties, excepting for Ni21 and Ni27 in stalk diameter. Meanwhile, NiF8 showed the lowest values for both stalk weight and diameter.

The differences among sugarcane varieties were significant in leaf dry weight, stalk dry weight, total dry weight, total N uptake, and TNUE, but not significant in SPAD (Table 5.2). In fact, NiF8 had the highest SPAD which significantly higher than NiF3, Ni17, Ni25, Ni27, Ni28, and Ni29. NiF8, Ni9, and Ni27 showed the highest values for leaf dry weight, significantly higher than Ni12, Ni15, Ni21, and Ni25. Meanwhile, NiF3 and Ni17 showed the highest values for stalk dry weight, significantly higher than Ni12, Ni15, N21, Ni25, and Ni29. The total dry weight of NiF3 was highest, which followed by Ni27 and NiF8, significantly higher than Ni12, Ni15, Ni17, Ni21, and Ni25. Ni27 and NiF3 showed the highest total N uptake values, significantly higher than Ni21 and Ni25. NiF3 also showed highest NUE, significantly higher than other varieties excepting for NiF8, Ni17, and Ni27.

There were significant positive correlations among target agronomical and growth parameters, excepting for stalk diameter ($r=0.16^{ns}$) with total dry weight. Moreover, biomass components including leaf and stalk dry weight had also positive correlations with total dry weight with coefficients of r=0.88** and r=0.98**, respectively (Table 5.3). The positive correlation was also found in the relationship between TNUE and total dry weight (r=0.69**).

Table 5.1. Tillering date, leaf area, tiller number, stalk height, single talk fresh weight and stalk diameter of sugarcane varieties under water stress conditions

Varieties	Tillering date (DAT)	Leaf area (m^2)	Tiller number plant^{-1}	Stalk height (cm)	Single stalk weight (kg)	Stalk diameter (cm)
NiF3	37	2.17^{abc}	4.3^{bc}	260.5^{a}	1.23^{a}	2.6^{a}
NiF8	30	2.53^{a}	8.0^{a}	248.2^{abc}	0.47^{e}	1.5^{e}
Ni9	32	2.20^{ab}	5.7^{b}	233.4^{b-e}	0.73^{cd}	2.0^{d}
Ni12	35	1.87^{bcd}	4.3^{bc}	220.4^{de}	0.80^{cd}	2.2^{bcd}
Ni15	36	1.40^{d}	4.7^{bc}	216.7^{e}	0.73^{cd}	2.1^{cd}
Ni17	34	2.00^{a-d}	4.3^{bc}	224.0^{b-e}	0.90^{bc}	2.3^{b}
Ni21	42	1.57^{cd}	3.7^{c}	225.1^{b-e}	0.87^{bcd}	2.4^{ab}
Ni22	31	1.93^{a-d}	5.0^{bc}	236.7^{a-d}	0.70^{d}	2.0^{d}
Ni25	36	1.63^{bcd}	4.0^{c}	223.2^{cde}	0.77^{cd}	2.2^{bcd}
Ni27	28	2.17^{abc}	5.0^{bc}	250.9^{ab}	1.03^{b}	2.4^{ab}
Ni28	31	2.00^{a-d}	5.0^{bc}	244.3^{a-d}	0.86^{bcd}	2.2^{bcd}
Ni29	26	1.53^{d}	5.0^{bc}	223.5^{cde}	0.90^{bc}	2.3^{bc}
CV%	-	19.3	19.1	6.8	13.5	6.3
Significance	-	*	**	*	**	**

* and ** mean non-significant, significant at $P < 0.05$ and $P < 0.01$, respectively. Different small letters in the same column show significance among sugarcane varieties at the same water levels at $P < 0.05$ by LSD.

Table 5.2. SPAD, stalk dry weight, leaf dry weight, total dry weight, total nitrogen uptake (TNU), and nitrogen utilization efficiency (TNUE) of sugarcane varieties under water stress conditions

Varieties	SPAD	Stalk dry weight (g plant^{-1})	Leaf dry weight (g plant^{-1})	Total dry weight (g plant^{-1})	TNU (g plant^{-1})	TNUE (g/g)
NiF3	42.2^{c}	585.3^{a}	201.1^{ab}	786.4^{a}	4.0^{ab}	197.6^{a}
NiF8	48.5^{a}	472.1^{abc}	238.5^{a}	710.6^{abc}	3.7^{abc}	190.6^{ab}
Ni9	44.6^{abc}	418.0^{ab}	234.5^{a}	$652.5^{a\text{-}d}$	3.8^{ab}	170.6^{cde}
Ni12	44.8^{abc}	371.8^{bc}	172.0^{b}	$543.8^{c\text{-}f}$	3.1^{bcd}	$175.8^{b\text{-}e}$
Ni15	45.9^{abc}	353.5^{c}	154.0^{b}	507.5^{def}	3.0^{bcd}	167.3^{cde}
Ni17	42.9^{c}	383.7^{abc}	212.4^{ab}	$596.0^{b\text{-}f}$	3.2^{bcd}	184.6^{abc}
Ni21	44.2^{abc}	276.6^{c}	160.0^{b}	436.6^{f}	2.8^{cd}	156.1^{e}
Ni22	48.0^{ab}	434.7^{abc}	203.5^{ab}	$638.2^{a\text{-}e}$	3.7^{abc}	$173.8^{b\text{-}e}$
Ni25	42.9^{c}	302.5^{c}	157.1^{b}	459.6^{ef}	2.7^{d}	166.1^{cde}
Ni27	43.1^{c}	528.3^{a}	238.9^{a}	767.2^{ab}	4.3^{a}	180.2^{abc}
Ni28	43.0^{c}	405.4^{abc}	197.8^{ab}	$603.2^{a\text{-}f}$	$3.5^{a\text{-}d}$	$171.4^{b\text{-}e}$
Ni29	43.6^{bc}	410.3^{bc}	205.5^{ab}	$615.8^{a\text{-}f}$	3.8^{ab}	162.9^{de}
CV%	5.9	19.5	18.6	18.7	16.15	6.75
Significance	ns	**	*	*	*	*

ns, * and ** mean non-significant, significant at $P < 0.05$ and $P < 0.01$, respectively. Different small letters in the same column show significance among sugarcane varieties at the same water levels at $P < 0.05$ by LSD.

Table 5.3. Correlations of agronomical and growth parameters with biomass production

(n=36)

Parameters	Total dry weight
Stalk number	0.54**
Stalk height	0.56**
Stalk diameter	0.16^{ns}
Stalk weight	0.56**
Leaf area	0.83**
Leaf dry weight	0.88**
Shoot dry weight	0.98**
TNUE	0.69**

ns, * and ** mean non-significant, significant at $P < 0.05$ and $P < 0.01$, respectively.

5.4. Discussion

In general, drought stress reduces sugarcane growth particularly in the reductions of plant height or plant elongating, leaf area or leaf dimension because of lacking energy and materials which support cell division and elongation processes when plant subjects to water deficit conditions. Many previous studies demonstrated the harmful effects of drought stress on plant height, plant elongating rate (Barbosa et al., 2015; Begum and Islam, 2012; Ethan et al., 2016; Chapter 2.1; Zhao et al., 2010); leaf number, leaf area or leaf area index (Barbosa et al., 2015; Begum et al., 2012; Chapter 2.1; Robertson et al., 1999), and SPAD (Chapter 2.1; Jangpromma et al., 2010; Silva et al., 2007; Zhao et al., 2010). In this study, the decrease in plant height increasing rate was recorded during the most water deficit period of 81 to 120DAT. This led to a lower plant height of stress treatment in comparison to that of control treatment from 81DAT until the end of the experiment. SPAD seemed to be less sensitive to water stress than plant height, which lower SPAD of stress treatment was observed in later period from 102 to 112DAT, then becoming similar to SPAD of control treatment from 123DAT (Figure 5.2). However, the difference in plant height and SPAD after the experimental period between two water regimes was not significant. The previous study suggested that starting irrigation when pF increasing to 2.8 may avoid plant growth reduction (photosynthetic activities) (Chapter 4). Therefore, drought stress (totally around 20 days) in this study was too short to have any significant impact on the sugarcane varieties' growth. Moreover, the disturbance from rainfall, especially during the later period of the experiment may give sugarcane plant the chance to recover. Jangpromma et al. (2010), the study in Chapter 2.2 also found the recovery of SPAD after drought stress period. Jangpromma et al. (2012) agreed that short-term drought stress (from 90 to 100 days after planting) did not have any noticeable effect on the relative rate of plant height growth of sugarcane.

In this study, variation in growth, yield component, biomass as well as NUE was found among investigated varieties. Genetic variation in leaf area, yield components, partial and total biomass were also found among sugarcane varieties by Basnayake et al. (2012); in Chapter 3; Jackson et al. (2016); Li et al. (2017); Luo et al. (2014); Ramesh (2000). The evidence of variation in NUE traits of sugarcane varieties was reported in Chapter 3; Ranjith and Meinzer (1997); Robison et al. (2009); Robinson et al. (2014) and Schumann et al. (1998). In this study, total dry matter accumulation had positive correlations with stalk number, stalk height and stalk weight (Table 5.3). Silva et al. (2008) and Tena et al. (2016) also found positive correlation among yield components (stalk number, stalk height, stalk diameter and stalk weight) and productivity under water stress and normal irrigation conditions. It suggested that sugarcane with high stalk number, stalk height, stalk weight and leaf area would be potential to get high dry matter accumulation. Moreover, because of the correlation coefficients of stalk number, and stalk weight with total dry weight were almost similar (r= 0.54** and r= 0.56**, respectively), the difference in stalk types (stalk weight and stalk number) did not have any noticeable effect on differences in total dry weight among varieties. This point of view is in line with Ehara et al. (1994). In this study, both leaf dry weight and stalk dry weight directly contributed total plant dry weight with higher correlation coefficient with the total dry weight of stalk dry weight (r= 0.98**) than that of leaf dry weight (r= 0.88**). It means that stalk has a larger contribution to total biomass production than leaf did. Furthermore, the finding in this study that there was a positive correlation between TNUE and total dry matter accumulation. It is line with Acreche (2017), and Calif and Edgecombe (2015) when they found positive associations between NUE traits with biomass production, sugar and cane yield under well-irrigated conditions. It suggested that higher NUE traits could support better biomass and yield performance of sugarcane under irrigated and rain-fed conditions. From this study, NiF3, NiF8, and Ni27 could be introduced as the promising varieties for higher NUE performance under rain-fed conditions.

CHAPTER VI
GENERAL DISCUSSION

More than 50% of sugarcane crop N is acquired during early growth stage. In sugarcane production, therefore, N is often applied several times from planting (basal dressing) until early elongating phase (top dressing) to support crop requirement. However, during this stage, high N loss is always implicit. Water deficit, which frequently occurs in this stage is one of the reasons for increasing N loss which followed by the deficiency of N uptake and low NUE, and resulting in low productivity. Improving NUE, therefore, is more important than increasing N application rate to achieve a better cane yield and sugar yield, especially under water stress conditions. This study focuses on the response of sugarcane to drought stress and different N applied levels to elucidate the relationship between NUE and drought tolerant ability in sugarcane.

The findings showed that photosynthetic activities of sugarcane were diminished when plant subjected to drought stress at early growth stage (Chapter 2.1, Chapter 3 and Chapter 4). It concurs with previous studies by Barbosa et al. (2015); Du et al. (1996); Graça et al. (2010); Koonjah et al. (2006); Silva et al. (2007, 2011); Riberiro et al. (2013). At first, drought stress reduced the conductance of stomata leading to a reduction in the CO_2 source into leaf tissue. Beside, Rubisco and PEPCase activity, the main limiting factors for photosynthesis, decreased when sugarcane subjected to drought stress (Du et al., 1996; lower A/Ci initial slope in water stress treatment compared to that in well-watered treatment shown in Chapter 2.1). The shortage of substrate and limitation of enzyme activity led to the reduction of the photosynthetic rate. The decline of photosynthetic activity depends on the levels of drought stress. Under mild stress, sugarcane plant could maintain photosynthesis at the high rate as under well-watered condition, but when water stress became more severe, photosynthesis was rapidly dropped and seemed not active when soil moisture content reaching to the permanent wilting point (Chapter 4, Zhao et al., 2013). However, photosynthesis of sugarcane is highly sensitive to water deficit, it is easy to reduce when soil moisture content decreases, but also easy to recover when re-watering, which could be found in Chapter 4. It could explain no difference was found in the photosynthetic rate of water stress and well-watered treatments in Chapter 2.2.

Lacking energy and products from photosynthesis leads to restricting cell division and elongation processes resulting in the reduction of sugarcane growth. The results in Chapter 2 and

Chapter 3 showed that drought stress at early growth stage reduced significantly the number of green leaves, leaf area, plant height, stalk diameter, the number of nodes and shoot root number. It consists with many previous studies in the negative effects of drought stress on sugarcane growth (Barbosa et al., 2015; Ethan et al., 2016; Robertson et al., 1999; Zhao et al., 2010). The decreases of source and sink (photosynthesis followed by vegetative tissue growth) lead to declining dry matter accumulations in both partial and the whole plant (Chapter 2 and Chapter 3). This result is in line with the previous studies in the reductions of leaves, stalk, root and total dry weights under the effect of drought stress at early growth stage (Barbosa et al., 2015; Zhao et al., 2010; Robertson et al., 1999; Wagih et al., 2003). In Chapter 5, although having particular effects on elongating rate and plant height, water deficit from the rain-fed condition was too short to reduce significantly the growth of sugarcane varieties. Maybe the disturbance from rainfall helped plant maintain acceptable photosynthetic activity; especially rain during the later period of the experiment may give sugarcane plant the chance to recover. In fact, no distinction in elongating rate was found between two water treatments (Figure 5.2). Jangpromma et al. (2012) agreed that short-term drought stress at early growth stage did not have any noticeable effect on the relative rate of plant height growth of sugarcane. It confirms the idea in Chapter 4 that starting irrigation should be done when pF increasing to 2.8 to avoid plant growth reduction.

Responses of sugarcane to N for photosynthetic activity and growth were investigated in Chapter 2. The higher photosynthetic parameters of N application treatments were the result of better activities of nitrogen reductase, Rubisco, and PEPCase compared tp 0N treatment when leaf nitrogen content was increased. It brought out higher growth namely in plant height, leaves number, leaf area, partial and total dry matter accumulation. Moreover, the larger root system did feedback to increase nutrient uptake following water absorption to support photosynthesis and above growth. This was claimed in positive correlations among photosynthetic rate, shoot, root and total biomass in Table 2.2. However, higher N application levels were not corresponding to higher growth, especially under the effect of drought stress. Under the well-watered condition, excepting for partial and total dry weight still increased, other parameters including photosynthetic rate, plant height, and leaf area did not have any significant change when N level increased over 180N. Under drought stress condition, there were no significant differences among dry matter traits of 180N and 270N treatment, whereas no significant increases of all other traits were found when N application dose was increased over 90N. Previous studies found the similar results that in normal, applying

N results in higher growth and yield (Rosa et al., 2015; Sime, 2013; Wiedenfeld, 1995; Wiedenfeld and Enciso, 2007); but in some case, higher N application does not have any significant effect when N application over the optimum level (Ishikawa et al., 2009; Koochekzadeh et al., 2009; Madhuri et al., 2011; Muchovej and Newman, 2004; Otto et al., 2014).

Genetic variations in A_{max}, leaf area, plant height, above parts, root, and total biomass were found among sugarcane varieties (Basnayake et al., 2012; Jackson et al., 2016; Li et al., 2017; Luo et al., 2014; Ramesh, 2000). In this study, the differences among the growth of sugarcane varieties were not clear in A_{max}, but clear in other growth and dry matter parameters under the drought stress and well-watered conditions in the pot experiment (Chapter 3) and the rain-fed condition on the field experiment (Chapter 5). Under the pot condition, NiF3 showed the best performance in growth and biomass among investigated varieties. Under field condition, it together with NIF8 and Ni27 also presented better growth and total biomass than other varieties. In general, genotype that better tolerance to drought stress maintains higher productivity, yield components, cane, and sugar yield (Begum and Islam, 2012; Hemaprabha et al., 2013; Ribeiro et al., 2013, Silva et al., 2008). It also has the tendency to develop deeper and larger root system (Endres et al., 2010; Ferreira et al., 2017; Jangpromma et al., 2012; Smith et al., 2005; Wagih et al., 2003). In Chapter 3, drought stress at early growth stage reduced total biomass of investigated varieties by from 22.8 to 31.4%. Among these varieties, NiF3 and Ni17 showed the lowest reduction rates. It could be because these two varieties had better drought tolerant ability to maintain high shoot and root as well as total biomass under drought stress conditions.

NUE depends on N application levels, various varieties, and soil water status. In normal, at the optimum N dose, the plant often has the highest NUE as well as cane and sugar yield (Koochekzadeh et al., 2009; Saleem et al., 2012). However, because of N deficiency, higher NUE at lower N levels somewhat are not synonymous with higher cane and sugar yield in comparison with lower NUE at higher N levels. Thorburn et al. (2014) reported that NUE reduced when the dose of N application was increased. Ranjith and Meinzer (1997) also found an increase of PNUE under N stress condition. Similarly, in this study PNUE was noticeable higher in N added treatments compared to 0N treatment. However, PNUE declined but not significantly, whereas BNUE significantly decreased along with the increase of N application doses from 90N to 270N (Chapter 2). The reduction of NUE could be the result of lower nitrate reductase activity, an important enzyme participates in N assimilation and remobilization processes, when sugarcane subjected to drought stress (Abayomi, 2001). In this study, the same results were found that water stress caused significant reductions in all NUE traits (Chapter 2, Chapter 3). Evidence for different NUE among sugarcane varieties was reported by Hajari et al. (2015), Robinson et al. (2009) and Schumann et al. (1998) under *in vitro*, glasshouse, and experimental field conditions, respectively. In this study, the genetic variation of target varieties was also found in NUE traits under both pot and field experiments with different soil water statuses i.e. well-watered, severe drought stress (Chapter 3), and rain-fed conditions (Chapter 5).

The main findings in this study reveal the relationship between NUE traits and drought tolerant ability (DTI). In fact, investigating with different N application levels, PNUE and BNUE had strong positive correlations to drought tolerant index with coefficients of r= 0.90** and r= 0.99**, respectively (Chapter 2). With different sugarcane varieties, high correlation coefficients were also found among PNUE (r= 0.66**), TNUE (r= 0.58*) and BNUE (r= 0.76**) with drought tolerant index (Chapter 3). It demonstrated the hypothesis that improvement of NUE under drought stress condition could be a solution to rescues sugarcane production under drought stress or better NUE traits could be the strategy to sugarcane varieties confront to drought stress at early growth stage. Therefore, NUE could be an added tool along with yield components (Silva et al., 2008) to screening drought tolerant sugarcane variety in future sugarcane breeding. Moreover, the positive correlation between TNUE with biomass production means that higher NUE trait supported better growth and dry matter accumulation performance in sugarcane varieties under rain-fed conditions (Chapter 5). Under pot conditions, NiF3 showed the best growth performance, TNUE as well as having better drought tolerant ability than other varieties. Under field conditions, it together with NiF8 and Ni27 also presented better growth and high NUE. NiF3 is also evaluated as a strong drought tolerant variety in Japan (NARO, http://www.gene.affrc.go.jp). Unfortunately, this variety is also considered as weak tolerant variety to the typhoon (Takagi et al., 2005), important abiotic stress in the tropical area. Therefore, it could be recommended to use as a reference variety and parental material in the breeding program for better drought tolerant ability at early growth stage.

In conclusion, sugarcane growth, biomass, sugar yield, and NUE traits reduced when plant subjected to drought stress at early growth stage. Changing of photosynthetic parameters were corresponding to the change of soil moisture or levels of water deficit. The photosynthetic parameters could be the indicators to determine the irrigation schedule. Applying N supported better physiological and agronomical performances, but increasing N was not often synonym with higher growth and NUE, especially under drought stress condition. Improvement of NUEs could help the plant to confront to drought stress. Selecting the optimum N dose to satisfy both better growth and NUE to avoid any risk from drought stress, therefore, is necessary. Moreover, sugarcane varieties with higher NUE traits had better growth, biomass, and drought tolerant ability. NUE traits should be used as an indicator to screen drought tolerant variety. The varieties, NiF3, NiF8 and Ni27 performed better growth and high NUE under rain-fed condition. These varieties could be introduced as the promising varieties in the breeding program as the reference or breeding materials for higher NUE performance and drought tolerant ability.

In this study, it is considered that better drought tolerant ability could help plant has stronger vigor to survive and maintain growth and biomass throughout water stress condition as high as at full irrigation condition. It is the premise for better recovery after stress period and results in higher yielding at the later stages. In normal, in the breeding program, yield components at harvest are checked to evaluate drought tolerant ability of sugarcane varieties. In my point of view, drought tolerant ability could detect earlier from early growth stage using NUEs as screening indicators. In this study, drought tolerant ability was evaluated by the maintenance ability of sugarcane after a drought stress period. However, the recovery ability from the drought stress is also important. For future studies, it is necessary to conduct long-term experiments (both under glasshouse and actual field conditions) to evaluate the relationships among NUE and recovery ability, and with yield performance at harvest to demonstrate the hypothesis that NUE could be used as a key tool to early screen drought tolerant variety.

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