STUDIES ON CHARACTERISTICS OF PHOTOSYNTHESIS, GROWTH AND SUGAR ACCUMULATION IN SUGARCANE UNDER DROUGHT AND FLOOD CONDITION

乾燥および湛水条件下におけるサトウキビの光合成特性，成長および糖蓄積に関する研究

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ABSTRACT

Sugarcane (*Saccharum* spp.) is an economically important crop for sugar and bioenergy production in many tropical and subtropical countries. In Thailand, sugarcane production has dramatically increased as a result of zoning policy from the government that focus on introducing crop that is more technically and economically suitable on under-utilized and/or under optimized land. Moreover, high domestic price encourage farmer switching lands from rice to sugarcane. However, rising global temperature accompanied with changes in weather and climate, which results in more frequent and more severe drought and flooding, negative effects on sugarcane are often observed in Thailand. These studies consist with 2 experiments as 1) Effects of duration and combination of drought and flood condition on leaf photosynthesis, growth and sugar accumulation in sugarcane, that the objective of this study was to investigate the effects of various combinations of drought and flood of varying duration on sugarcane growth and yield during the grand growth phase 2) Changes in photosynthesis, growth and sugar accumulation of commercial sugarcane cultivars and *Erianthus* under flood condition that has the objective to determine changes in photosynthesis, growth and sugar content in three commercial sugarcane cultivars and *Erianthus* under long-term flood condition and after change to normal condition.

Experiment 1 investigated the individual and combined effect of drought and floods of varying duration on cultivar NiF8 growth under glasshouse condition. During 6 months, 6 treatments that set as drought for 15 days, prolonged drought for 30 days, flood for 15 days, prolonged flood for 30 days, flood followed by prolonged drought and prolonged flood followed by prolonged drought. Control and drought plants were irrigated an equivalent amount of 50 and 20%, respectively, whereas flood plants were submerged as high as 35 cm above the soil surface. The result demonstrated that CO_2 assimilation was reduced dramatically in sugarcane plants that were exposed to drought and prolonged drought treatments but recovered once the plants were re-irrigated. By contrast, the flood did not affect CO_2 assimilation, even when the water was drained, whereas combination treatments were reduced CO_2 assimilation when exposed to prolonged drought. During flooding, adventitious roots with well-developed aerenchyma were produced, the number of root increased under prolonged flooding, which may help plants to offset the losses associated with flooding. The expansion of leaf area was interrupted by drought and remained at a low level even after re-irrigation, while this increased once the flood and combination treatments had finished. The stem and total dry weight increased as a result of the flood and combination treatments but reduced during the drought treatments. At the time of harvest, there were no significant differences in stem fresh weight, sucrose content, or sugar yield between treatments.

Experiment 2 investigated the effects of floods on 3 sugarcane cultivars (NiF8, U-thong 6 (UT6), U-thong 9 (UT9)) and *Erianthus* spp. Growth was assessed with two treatments: 1) control and 2) 60 days of flooding followed by 30 days of normal conditions. Control plants was irrigated an equivalent amount of 50%, whereas flood plants were submerged as high as 35 cm above the soil surface. The results revealed, in comparison with control, during prolonged flooding, *Erianthus* showed greatly decreased CO_2 assimilation, whereas NiF8, UT6, and UT9 showed slightly declined CO_2 assimilation. Growth in plants subjected to 60 days of flooding was less influenced by floods while sucrose content was not affected except in UT6. During

flooding, some roots died, resulting in plants compensating adventitious roots to offset the negative effects of root death and to assist them maintaining their growth, which appeared from the submerged nodes, with different characteristics for each cultivar. However, 30 days after draining, roots remained damaged, while adventitious roots died, resulting in a lesser growth as compared with the control. From this study, each of the sugarcane cultivars and *Erianthus* were thought to have their advantages to survive under a flood condition. Sugar yield, which is the most important factor for sugar refinery, was lowered by flooding in all the cultivars on 90 DAT. However, the decreases were not statistically significantly, suggesting flooding for 60 days at this growth stage had little effects on sugarcane.

In conclusion, the extent of damage by drought and flooding on sugarcane differs between the cultivars and duration of stress. This study demonstrated that drought was effects to sugarcane rapidly by limited plant mechanism resulting in reduction of growth. However, sugarcane able to recovery by re-irrigated if not severe drought. On the other hand, flooding had less effect to sugarcane may cause from the development of aerenchyma in adventitious root which may have helped the plants to compensate root death and maintaining their growth. After drained root remained damaged and not recovery, then un-recovery of root may result in reduction of growth and sucrose accumulation at post flood. Thus, flooded plants required time to develop their new roots and recover their growth.

However, results from both experiments that evaluated in glasshouse condition may different from the field experiment that the environment is varying. Then, my future study in Thailand, field study and field observation are need to study for more understanding the effects of drought and flood stress with various area and environment. In addition, the result demonstrated that plant still continued effect by drought and flood even plant was return to normal condition. Thus, the effect of post-drought and flood to yield and quality in plant cane and ratoon cane is important to study. Moreover, after drought or flood, management of soil, irrigation and fertilizer are necessary to study for more information of field management that benefit for plant growth and obtaining the optimum yield and quality at harvesting.

CONTENTS

CHAPTER 1

General Introduction

Sugarcane (*Saccharum* spp.) is an important economic crop for sugar industrial and bioenergy potential in tropical and subtropical countries. High sucrose content in cane is the first priority of sugarcane farmers and sugar mills to obtain high price and sugar yield, respectively. Furthermore, the residues of sugar mills can be developed as an alternative source of energy as biomass and bioethanol. Sugarcane provide a juice, which is used for making sugar that importance for food industrial. Sugar is important ingredient for various kind of foods, including the human appetite for sweet foods and drinks, the complementarity that sugar brings to the other flavors in food, used for preservation and fermentation propose (International Encyclopedia of the Social Sciences, 2008). Moreover, sugar is important for medical industrial for making and preserving various kind of medicines like syrups, liquids, capsules and etc. By product as molasses is used in distilleries for manufacture of ethyl alcohol, butyl alcohol, citric acid, rum that is the potable sprit, and used as an additive to feeds for livestock (Vinod, 2013). Thus, sugar is very important for human living and economic in many countries that planting sugarcane. However, due to an increasing of global population, demand of sugar would be increase while sugarcane production are facing to global climate change, result in extreme environment such as drought and flood cause of insufficient of sugar in the future. Then, cane grower should realize the effect of climate change and prepare to cope with the uncertain climate to their sugarcane.

1.1 Global sugar production and consumption

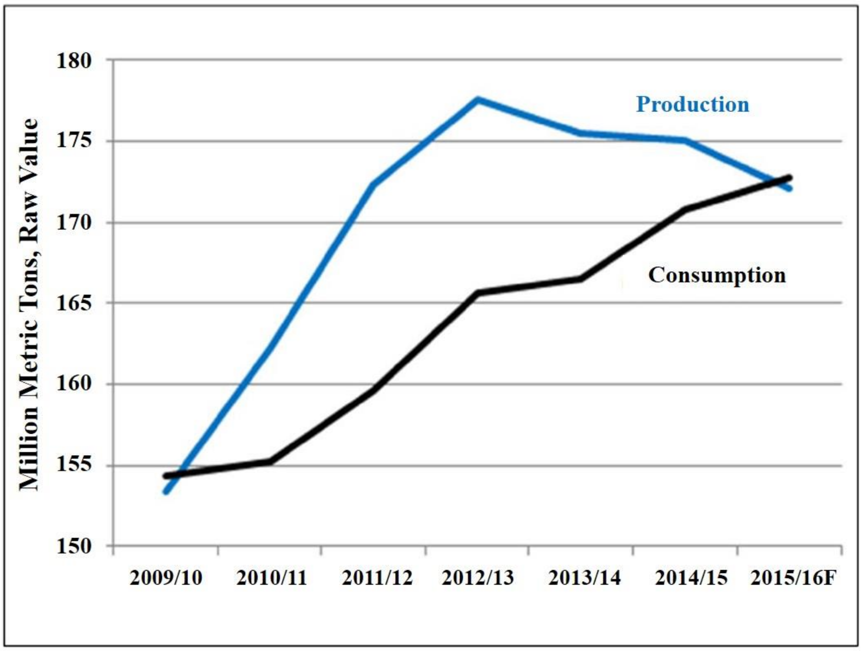

Fig. 1.1 Global sugar consumption outpaces production during 2009/10–2015/16. Source: USDA Foreign Agriculture Service (2015b).

Currently, global sugar consumption expands while production decelerate. The global sugar production has reduced since 2012/13 from 177 million metric tons to 173 million metric tons in 2014/15, whereas global sugar consumption increased from 165 million metric tons to 170 million metric tons. Moreover production for 2015/16 is forecast down 3 million metric tons (raw value) at 172 million metric tons while consumption is projected to reach 173 million metric tons (USDA Foreign Agriculture Service, 2015a). Due to the increased of population as well as economic growth are not only key to increase global consumption but also many staple foods. Moreover, rising standard of living usually lead to higher consumption of processed foods as dairy, soft drinks, foods and etc. which have high sugar content. Also, the so-called income elasticity of sugar demand is usually higher for low income countries than high income countries (Licht, 2015).

1.2 Sugarcane production in Thailand

Thailand is one of the world's top five producers, with more than 100 million metric tons of sugarcane production annually or more than 10 million metric tons of sugar produced in 50 sugar mills and become the second largest sugar exporter on the world sugar market (SUGAR Expertise, 2015). Thailand is number 5 of sugar producer' share of world total that 5.7% in 2012/13 and increased to 6.60% in 2016/2016. Exported sugar from Thailand is mainly in Asia country with 86.3% (42% ASEAN) of total export that more than 7 million metric tons. Main import countries form Thailand in 2014 is Indonesia (24%), follow by Japan (10%), China (10%), Malaysia (9%), Cambodia (7%) and etc. respectively (Pipat, 2015).

Sugarcane is cultivated in 1.5 million hectare with four main producing regions as North region (0.38 million hectares), Northeast region (0.7 million hectares), Central region (4.8 million hectares), and East region (0.9 million hectares). The total production is highest the North region at 60 million metric tons with average yield 73.1 metric tons per hectare, followed by Central region 32.5 million metric ton with 68.0 metric tons per hectare, North region 26.9 million tons with 69.7 metric tons per hectare and East region is 6.2 million metric tons with 69.7 metric tons per hectare (Office of Agricultural Economics of Thailand, 2014).

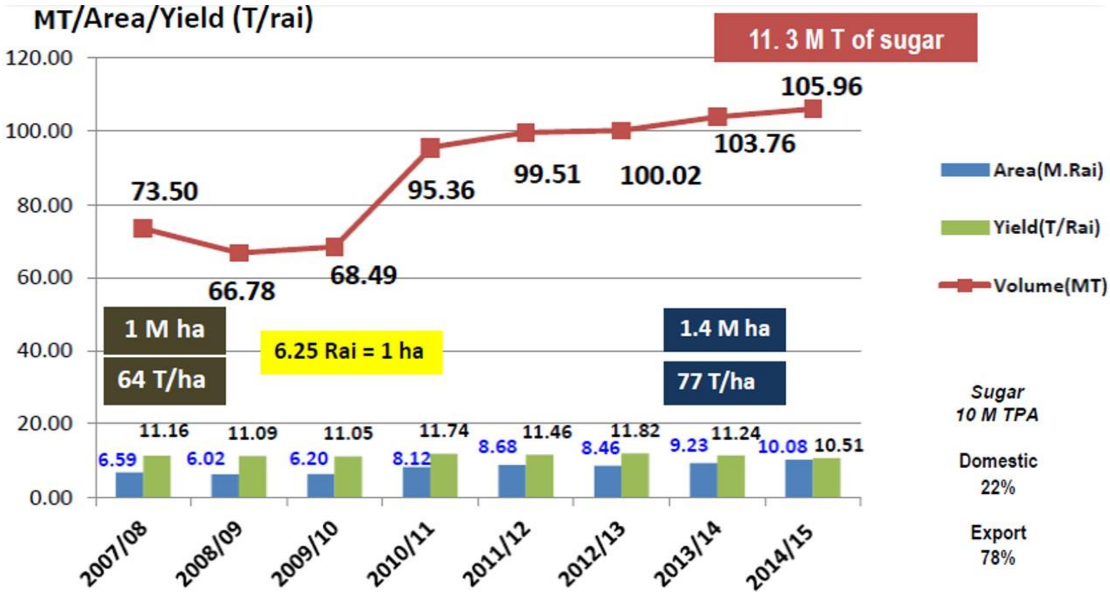

Fig. 1.2 Thai sugarcane production, yield (ton/rai) and production during 2007/08–2014/15 (Pipat, 2015).

Moreover, sugarcane production in 2015/16 is forecast to increase to 107 million metric tons with sugar production increased to 11.4 million metric tons due to area expansion (32,000 hectares) and the government incentives under the 5–year Agricultural Restructuring Program (2015/16–2019/20) to move area from rice to other crop as sugarcane, oil palm, corn and cassava (USDA Foreign Agriculture Service, 2015a).

1.3 Current situation of sugarcane in Thailand

Sugarcane is a major economic crop in Thailand, where production has dramatically increased as a result of the Government encouraging rice (*Oryza sativa*) producers to switch to sugarcane production for better returns (USDA Foreign Agriculture Service, 2014) and continue with 5–year Agricultural Restructuring

Program (2015/16–2019/20) to move area from rice to other crops (USDA Foreign Agriculture Service, 2015a). Besides, The Office of The Cane and Sugar Board under the Ministry of Industry has announced the strategic plan for sugarcane and sugar (2015–2026) to develop sugar industry in Thailand. Under this plan, sugarcane plantation is expect to increase up to 52.3% or 2.56 million hectare, sugarcane yield increase 69.8% or 180 million metric tons, average yield per area increased 13.3% or 71.25 metric tons/hectare, sugar production increase 79.5% or 20.36 million metric tons, efficiency increase 4.6% or 112 kg/ton of sugarcane, ethanol production increase 115.2% or 5.38 million litter/day, and produce electricity increase 159.4% or 4,000 MW in 2026 (Table 1.) (Office of Cane and Sugar Board, 2016).

	2015	2026
Plantation	1.68 million hectare	2.56 million hectare
Sugarcane yield	105.96 million metric tons	180 million metric tons
Average yield per area	62.87 metric tons/hectare	71.25 metric tons/hectare
Sugar production	11.34 million metric tons	20.36 million metric tons
Efficiency	107.02 kg/ton of sugarcane	112 kg/ton of sugarcane
Ethanol	2.5 million litter/day	5.38 million litter/day
Electricity	1,542 MW	4,000 MW

Table 1. Strategic plan for sugarcane and sugar 2015–2026 of Office of The Cane and Sugar Board, Ministry of Industry, Thailand.

Source: Office of The Cane and Sugar Board, Ministry of Industry, Thailand.

Then, sugarcane production is expected to increase production and export expansion in the future due to the stable returns compared to other crops and cane grower are getting support from the government for soft loans and input the subsidies.

However, an increase of sugarcane production in Thailand may from increase of plantation, while yield per area is decreased due to unfavorable weather condition.

1.4 Sugarcane cultivation in Thailand

In Thailand, the planting patterns are depend on the topography and climate of the area. However, there is only 10% of plantation under irrigation, most of sugarcane plantations are still relying on rain-fed systems, which present challenges for water management. Sugarcane farmers in the Northeast region generally plant their cane in October and November, and in the Eastern Central Plains region, November to February. Planting in the irrigated area of the North region is December to April and May to June in the rain-fed area. In the Western Central Plains area, planting in irrigated areas is from January to March and in the rain-fed area May to June. While the sugarcane crop calendar varies by region, the growing period is about 10 to 14 months depending on the variety of cane (FAO CORPORATE DOCUMENT RESPOSITORY, 1997). Usually, sugarcane was harvesting during November to March but due to an increase of sugarcane production result in an expansion of harvesting period. However, Thai sugar producer still have problems with uncertainly of climate cause in extreme of weather as drought and flood effect to growth, yield and quality of sugarcane. Lack of mechanization and small farm size lead to increase of labor intensive result in high cost of production.

1.5 Global climate changes and effect to agriculture

Global climate change caused by natural processes and anthropogenic factor result in effects of major environmental and will continuously affect to agriculture. Atmospheric CO_2 concentration has increased about 30% since the mid of 18th century due to increases in combustion of fossil fuels, transportation, rapid developed of industrial and deforestation (Duli and Yang, 2015). Global warming is directly associated with increasing atmospheric CO_2 concentration and other greenhouse gases (GHG). Global, surface mean temperatures had increased from 0.55 to 0.67 ºC in the last century and are project to rise from 1.1 to 2.9 ºC (low emission) or 2.0 to 5.4 ºC (high emission) by 2100 relative to 1980–1999, depending on GHG emission level, region, and geographic location (IPCC, 2014). Climate variability and climate change are projected to result in changes in sea levels, rainfall pattern, and the frequency of extreme high and low temperature events, floods, droughts, and other biotic stress become more frequent and more intense in many part of the world (Dhillon and Wuehlisch, 2013; Trenberth et al., 2007). Climate change was negatively impacts to agricultural production and cause breakdown in food system and lead to food insecurity (World Health Organization, 2015).

Thailand is the home of 67 million people, agriculture employs 49% of the population and contributes 10% of GDP. Due to the climate change threatens, 3 majors sector of Thailand’s economy were affect as agriculture, tourism, and trade. Now a day, Thailand produce 0.8% of the world’s carbon emissions and lower than average rate of capital emission of the global average. However, Thailand’s total CO2 emission was double between 1991 and 2002 and government recognized it contribution to global warming. The effects of climate changes are appear as increased of surface temperature, frequency of flood and drought, rainfall pattern, severe storm and increased of sea level. Erratic weather lead Thailand become drought and flood with severe, more frequent and unpredictable. The weather patterns in Thailand have fluctuated from severe droughts to severe flood affect to agricultural areas. Changed of weather caused in low rainfall in 1993 result in drought and water shortage, whereas intense rainfalls in 1994, 1995, 2011 result in the worst floods. In 2005, 11 million people in 71 provinces were affected by water shortage, while in 2008 severe drought affected to people and agriculture (Corinne, 2008).

1.6 Climate change and possibility effect to sugarcane in Thailand

Due to rising global temperature accompanied by changes in weather and climate, which result in more frequent and more severe drought and flooding, negative effects to sugarcane are being experienced, particularly during the rainy season. In Thailand, rainy season are start from May to September, there are two rainfall peaks in May and August, and a dry spell in July (Roongroj and Long, 2006). Consequently, in the worst case, plants could suffer from both flood and drought during a single rainy season. Furthermore, the change from paddy field to sugarcane production may increase the risk of floods and cause problems for cane growers, particularly in the central basin. Moreover, most of sugarcane area in Thailand are planting in rain-fed area, only 10% of area are under irrigation. Then, water management is need improve to develop growth and yield of sugarcane.

Usually, two predominant sugarcane planting patterns exist: 1) at the end of the rainy season (October–January) in an irrigate area and 2) in the early part of the rainy season (April–May) in rain-fed areas. Sugarcane has four growth phases: germination and emergence (1 months after planting); tillering and canopy establishment (at 2–3 months); grand growth (at 4–10 months); and maturation or ripening (at 11–12 months) (Gascho, 1985). During the rainy season, sugarcane is 5–6 months of age and in the grand growth phase, which is important for actual cane formation, elongation, and yield build up. Thus, drought and flood at this time may affect growth and, thus, the yield and quality of sugarcane produced during the maturation or ripening phase. However, the severe of effects from drought and flood to sugarcane is depend on genotype, stage of growth, duration of stress, soil type and environment condition.

1.7 Previous study on effects of drought and flood to sugarcane

1.7.1 Effect of drought to sugarcane

Effect of drought to photosynthesis

Drought or a limited water supply usually suppresses the rate of assimilation and leaf extension, and promotes leaf senescence (Inman-Bamber, 2004; Smit and Singels, 2006). CO_2 assimilation and the transpiration rate were dramatically reduced when plants were subjected to drought due to the stomata closing to prevent transpiration loss, which reduced the amount of CO_2 required for photosynthesis (Koonjah et al., 2006; Cornic and Massacci, 1996). Previous study on severe drought, which showed that stomatal conductance and water use efficiency declined by 5% and 7% of the control, respectively (Joseph and Leon, 2009).

Effect of drought to root and growth

Drought reduced the root dry weight, the hypocotyl length, and the fresh and dry masses of shoots and roots of alfalfa (*Medicago sativa*) decreased under water deficit (Zeid and Shedeed, 2006). Roots that grow in drought conditions exhibit profuse growth and are relatively longer with thin rootlets (Hidaka and Karim, 2007). Plants that were exposed to drought experienced a reduction in growth, water deficit resulted in a reduction in turgor pressure, the interruption of water flow from the xylem to the surrounding elongation cells, and a slowing down of the growth process, particularly in terms of a decrease in cell elongation and cell volume, an increased concentration of cell sap, and the progressive dehydration of the protoplasm (Larcher, 2001; Nonami, 1998). Low biomass accumulation under water stress has been attributed to a reduction in light interception, plant extension rate, and photosynthesis (Koonjah et al., 2006). Drought that occurs when the leaf canopy is well established will have more serious impacts on total biomass, stalk biomass, and stalk sucrose levels at harvest than drought that occurs in younger crops (Robertson et al., 1999a). Drought stress during the grand growth phase leads to variable reductions in cane and sugar yield depending on the growth stage but a constant reduction in sucrose content (Wiedenfeld, 2000). During the grand growth phase, tiller growth and development occur, alongside height gain and basal sugar accumulation, and so this is known to be a critical stage for drought sensitivity due to plants requiring large amounts of water for growth (Sonia et al., 2012; Ramesh and Mahadevaswamy, 2000).

Effect of drought to sucrose content

The sucrose content of the sugarcane juice increased during the early stages of drought but then decreased under prolonged drought conditions, sucrose content increased during a period of low rainfall or under dryland conditions but was reduced under extreme drought conditions (Robertson et.al., 1999a). Moreover, there is a reduction in stalk elongation as a result of low rates of leaf development and photosynthesis, which leads to low cane and sucrose yield (Robertson et al., 1999b), whereas 6 weeks drought during the grand growth phase was found to reduce the sucrose content by an average of 4.7% and sugar yield by 11.7%–19.1% (Wiedenfeld, 2000).

1.7.2 Effect of flood to sugarcane

Effect of flood to photosynthesis

Under flood condition CO_2 assimilation decrease because of the slow diffusion of CO_2 in water and the decreased availability of light, resulting in a decreased flow rate of assimilates to the root. The decreasing of CO_2 assimilation was dependent on many factors such as genotypes, environmental conditions, stage of growth, and duration of stress (Gomathi et al., 2014). It has been found that the application of periodic flooding every months leads to a 50% reduction in the photosynthesis rate (Viator et al., 2012). However, several study reported the neutral or positive effect of flood to sugarcane photosynthesis as the effect of the 7 days periodic cycle (during Feb–Aug) with different cultivars showing an unaffected CO_2 assimilation to Ho 01-12 (energy cane), HoCP 96-540 (sugarcane), and L99-226 (sugarcane), whereas affecting L79-1002 (energy cane) by being decreased by 50 and 48% in plant cane and ratoon cane, respectively (Viator et al., 2012). Previous study that reported a neutral or positive response of CO_2 assimilation, Transpiration, and stomatal conductance to periodic 7 days flooding (Glaz et al., 2004a), whereas 41 d of flooding decreased CO_2 assimilation, but increased stomatal conductance in comparison with the control (Hidaka and Karim, 2007). Furthermore, the periodic flood cycle found a neutral or positive response under a flooded condition, as plants continued transpiration (Chabot et al., 2002)

Effect of flood to root and develop of adventitious root

After flooding, the immediate effect is the absence of oxygen, and a change from aerobic to anaerobic environment affects the growth and functions of roots. Root hairs die and eventually blacken and rot, resulting in the entire underground root being choked, and root respiration also being impaired, thereby affecting important metabolic activities of plants (Gomathi et al., 2014). However, the plants concurrently develop adventitious roots from the nodes above the soil (Begum et al., 2013). These roots developed as a result of the hormonal imbalance that is induced by hypoxia in the submerged tissue, and were located in the upper layer of water, which has higher oxygen content (Gomathi et al., 2014). Three types of roots were produced after flooding: the first type emerged from the nodes that were located under the water, the second type developed from these first roots and grew upward against gravity, and the third type emerged from aerial nodes above the water (Hidaka and Karim, 2007). Adventitious roots with well-developed aerenchyma help plant to maintenance of root activity and the supply of required oxygen, and also contribute to higher dry matter accumulation (Drew, 1997; Gilbert et al., 2007; Gomathi et al., 2014).

Effect of flood to growth and yield

Cane and sugar yield were decreased because of decrease in photosynthesis, root development, leaf area (LA), LA index (LAI), tiller production, stalk height, and sucrose yield (Gomathi et al., 2014; Viator et al., 2012; Webster and Eavis, 1972). Flooding inhibited leaf expansion and decreased LA, LAI, and leaf weight (Gilbert et al., 2007; Gomathi et al., 2014) however, whereas flooding for less than 3 months may be less damaging to LAI (Gilbert et al., 2008). Waterlogging over 15–60 days over the grand growth phase decreased yield by approximately 5–30% because of the lack of nutrition and water uptake (Gomathi et al., 2014), while 3 months of flooding decreased the yield by 18–37% in plant cane and 61–63% in a second ratoon (Gilbert et al., 2008). However, growth and yield loss may depend on the tolerance of the cultivar, as it has been shown that there is a loss in yield in CP 95-1376 but not in CP 95-1429 because of exposure to 7 days of periodic flooding at water table depths of 16, 33, and 50 cm (Glaz et al., 2004b), whereas high water table had no effect on yields of CP 72-2086 and CP 82-1172, whereas the effect to CP 80-1743 resulted in a decrease in yield by 25.1% (Glaz et al., 2002). Furthermore, the study on 7 days periodic flooding found biomass yield of L79-1002 (energy cane) was not affected, while the biomass yield of Ho 01-12 (energy cane), HoCP 96-540 (sugarcane), and L 99-226 (sugarcane) in plant cane was affected. However, in ratoon cane, both energy canes were not affected, whereas both sugarcanes were affected by flooding (Viator et al., 2012).

Effect of flood to sucrose content

Previous study reported the positive effect of flood as 2 days of periodic flooding in each of eight 14 days cycles increased cane and sucrose yield in the CP 72-2086 and CP 80-1827 cultivars (Glaz and Gilbert, 2006). Furthermore, energy cane such as L79-1002 and Ho 01-12 were not affected by 7 days of periodic flooding; by contrast in sugarcane such as HoCP 96-540 and L 99-226, sucrose yield was decreased by 16 and 38%, respectively (Viator et al., 2012) whereas, sugar yields were not affected in CP 72-1210 when grown in a high water table (Pitts et al., 1990).

1.8 Essential research to studies the individual and combine effect of drought and floods to sugarcane

Sugarcane is important economic crop in Thailand, where production has dramatically increased as a result of the high price and better return than other crop. During the past decade, weather pattern in Thailand have fluctuated due to the global climate change result in extreme environment, such as high surface temperature, change in pattern of rain result in severe drought and floods and effect to the economic especially in agricultural sector. Most of agricultural area are still under rain-fed area then crops were risk to both drought and flood in every year result in low yield and quality. However, for sugarcane, high sucrose content in cane is the first priority of sugarcane farmers and sugar mills to obtain high price and sugar yield. Then, how to obtain an optimal yield and quality at harvesting even under uncertain of climate is need to study.

Thus, study on effects of duration and combination of drought and flood including with study in different varietal of sugarcane and *Erianthus* may advantage for cultivation, water management and sugarcane breeding in the future.

To the best of our knowledge, no previous study on sugarcane has examined the combined effects of flood and drought and information regarding physiological characteristics and growth ability of commercial cultivars and *Erianthus* under a flooded condition is lacking. The objective of this study were;

1) To investigate the effects of various combinations of drought and flood of varying duration on sugarcane growth and yield during the grand growth phase. In addition, morphological and chemical changes in the sugarcane juice and adventitious roots were analyzed to gain a better understanding of their adaptive significance.

2) To determine changes in photosynthesis, growth, and sugar accumulation in three commercial sugarcane cultivars and *Erianthus* under long-term flood conditions and after the change to normal conditions. Moreover, morphological changes and

development of adventitious roots were analyzed for gaining a better understanding of its adaptive significance of tolerance in each cultivar.

CHAPTER 2

Effects of Duration and Combination of Drought and Flood Conditions on Leaf Photosynthesis, Growth and Sugar Accumulation in Sugarcane

2.1 Abstract

Global climate change will result in extreme environments, such as droughts and floods. We investigated the individual and combined effects of droughts and floods of varying duration on sugarcane (*Saccharum* spp.) growth using a pot experiment under glasshouse conditions with the following six treatments: drought for 15 days, prolonged drought for 30 days, flood for 15 days, prolonged flood for 30 days, short flood followed by prolonged drought, and prolonged flood followed by prolonged drought. Plants that were subjected to drought conditions, including drought after a flood, had reduced CO_2 assimilation (through stomatal closure) and leaf areas, whereas flood conditions showed no effect. During flooding, some roots died, and adventitious roots with well-developed aerenchyma appeared from the submerged nodes. At the time of harvest, there were no significant differences in stem fresh weight, sucrose content, or sugar yield between the treatments. However, ion content analysis revealed that flood conditions caused an accumulation of sodium in the bottom of stems and adventitious roots. Therefore, under flood conditions, plants may develop adventitious roots, which may compensate the negative effects of root death, helping them to maintain their growth and yield.

2.2 Introduction

Sugarcane (*Saccharum* spp.) is a major economic crop in many tropical and subtropical countries, including Thailand, where production has dramatically increased as a result of the Government encouraging rice (*Oryza sativa*) producers to switch to sugarcane production for better returns (USDA Foreign Agriculture Service, 2014). In Thailand, there are two sugarcane planting patterns: 1) at the end of the rainy season (October–January) and 2) in the early part of the rainy season (April–May) with the selected pattern depending on the topography and climate of the area. However, most of sugarcane plantations are still relying on rain-fed systems, which present challenges for water management.

During the rainy season (May–September), there are two rainfall peaks in May and August, and a dry spell in July (Roongroj and Long, 2006). Consequently, in the worst case, plants could suffer from both flood and drought during a single rainy season. Furthermore, changing from paddy field to sugarcane production may increase the flood risk and cause problems for cane growers, particularly in the central basin during the rainy season. Sugarcane has four growth phases: germination and emergence (1 months after planting); tillering and canopy establishment (at 2–3 months); grand growth (at 4–10 months); and maturation or ripening (at 11–12 months) (Gascho, 1985). During the rainy season, sugarcane is 5–6 months of age and in the grand growth phase, which is important for actual cane formation, elongation, and yield build up. Thus, drought and flood at this time may affect growth and, thus, the yield and quality of sugarcane produced during the maturation or ripening phase.

The amount of flood stress experienced by sugarcane plants depends on the duration of waterlogging, the condition of the floodwater, and soil type (Gomathi et al., 2014). Both cane and sugar yield have been reported to decrease under flood conditions as a result of a reduction in photosynthesis, root development, leaf area, leaf area index, tiller production, stalk height, and sucrose yield (Gomathi et al., 2014; Webster and Eavis, 1972; Viator et al. 2012). It has been found that the application of periodic flooding every months leads to a 50% reduction in the photosynthesis rate (Viator et al., 2012) and reduced plant growth as a result of a decrease in the metabolic activity of the roots due to hypoxia (Gomathi et al., 2014). However, the plants concurrently develop adventitious roots from the nodes above the soil (Begum et al., 2013)–and when the flood lasts for 3 months, the sugarcane plants produce adventitious roots with well-developed aerenchyma, which may help plants to continue to take up water and nutrients (Gilbert et al., 2007; Gilbert et al., 2008).

Drought or a limited water supply usually suppresses the rate of assimilation and leaf extension, and promotes leaf senescence (Inman-Bamber, 2004; Smit and Singels, 2006). Roots that grow in drought conditions exhibit profuse growth and are relatively longer with thin rootlets (Hidaka and Karim, 2007). By contrast, there is a reduction in stalk elongation as a result of low rates of leaf development and photosynthesis, which leads to low cane and sucrose yield (Robertson et al., 1999b). Drought that occurs when the leaf canopy is well established will have more serious impacts on total biomass, stalk biomass, and stalk sucrose levels at harvest than drought that occurs in younger crops (Robertson et al., 1999a). Drought stress during the grand growth phase leads to variable reductions in cane and sugar yield depending on the growth stage but a constant reduction in sucrose content (Wiedenfeld, 2000). During the grand growth phase, tiller growth and development occur, alongside height gain and basal sugar accumulation, and so this is known to be a critical stage for drought sensitivity due to plants requiring large amounts of water for growth (Sonia et al., 2012; Ramesh and Mahadevaswamy, 2000).

To the best of our knowledge, no previous study on sugarcane has examined the combined effects of flood and drought. The objective of this study was to investigate the effects of various combinations of drought and flood of varying duration on sugarcane growth and yield during the grand growth phase. In addition, morphological and chemical changes in the sugarcane juice and adventitious roots were analyzed to gain a better understanding of their adaptive significance.

2.3 Materials and Methods

The experiment was conducted in a glasshouse at the University of the Ryukyus, Okinawa, Japan (26°15′N, 127°45′E; altitude 127 m). Sugarcane (*Saccharum* spp. cv. NiF8) seedlings were germinated in a tray on April 3, 2013, and transplanted in pots (1/2,000 a) filled with soil, sand, and peat (2:1:1, v/v). Initially, an automatic drip irrigation system was used to water the plants for 15 min (495 mL) every morning. The level of irrigation was then doubled at 3 months by also watering the plants at noon and tripled at 5 months through the addition of an evening watering. During growth, tillers were removed, and plants were kept in individual pots.

The experiment simulated a planting schedule that was similar to the Thailand pattern of planting in the early part of the rainy season (April–May). The flood and dry spell then occurred during the rainy season (May–September), when the sugarcane plants were 4–6 months of age, i.e., during the grand growth phase. At 6 months after transplanting (October 1), uniform plants were selected with an average sub-stem length of 263.5 cm. These plants were then subjected to a drought or flood. During the drought, plants received less irrigation, to an equivalent of 15% (v/v) soil moisture, whereas during the flood, plants were submerged up to 35 cm above the soil surface in 45-L plastic buckets. In total, six treatments with varying flood/drought durations and combinations were used along with a control, as follows: drought for 15 days, prolonged drought for 30 days, flood for 15 days, prolonged flood for 30 days, flood for 15 days followed by prolonged drought for 30 days (F + PD), prolonged flood for 30 days followed by prolonged drought for 30 days (PF + PD), and no flood or drought (control). In the combination treatments (F +PD and PF + PD), the plants were drained and then PD was applied by irrigating the plants at 15% (v/v) soil moisture for 30 days.

Each treatment included 18 plants for 6 times sampling with 3 replications each time. All plants were fertilized weekly by replacing the irrigation water with 500 ml of Hoagland's nutrient solution, which consists of 4 mM KNO_3, 6 mM $Ca(NO_3)_2·4H_2O$, 2 mM $MgSO_4·7H_2O$, 2 mM KH_2PO_4, 0.5 µM $CuSO_4·5H_2O$, 6.3 µM $MnSO_4·5H_2O$, 2 µM $ZnSO_4·7H_2O$, 25 µM H_3BO_3, 0.3 µM $Na_2MoO_4·2H_2O$, and 0.1 mM Fe(III)-ethylenediaminetetraacetic acid (EDTA). During the flood treatments, this solution was mixed into the floodwater. Plants were arranged with 40 × 90 cm spacing between the plants and rows in a completely randomized design.

Three plants from each treatment were sampled 1 days before the start of treatment (−1), and 15, 30, 45, 60, and 75 days after treatment (DAT). The leaf area

(LA) of the whole plant was measured with a leaf area meter (LI-3100, LI-COR). For stem sampling, stems were cut from the base, and the sub-stem length (distance from the soil level to the visible auricle of the top visible dewlap leaf (TVD)) and stem weight were measured. Each stem was evenly separated into bottom, middle, and top, and squeezed with a three-roller mill to obtain sugarcane juice from each part. For root sampling, adventitious roots appearing from the submerged stem were collected separately and dried in an oven at 80ºC for 48 hours to determine their dry weights. Adventitious roots were also sampled at 10, 20, and 30 DAT, by cutting them 1 cm above the tip and fixing them with formalin-acetic acid-alcohol (FAA) solution (50% ethanol:acetic acid:formaldehyde, 18:1:1). These sections were then viewed under light microscope and photographed (ECLIPSE 80i, Nikon). The juice was diluted 50 times with distilled water and filtered with a 0.45-μm membrane filter (ADVANTEC), following which the sugar content was analyzed with high-performance liquid chromatography (HPLC) (LC-20A, Shimadzu), and anion and cation contents were analyzed with ion chromatography (ICS-1600, Thermo Scientific). Sugar yield was calculated using the following equation:

$$\text{Sugar yield} = \text{sucrose content (\%)} / 100 \times \text{stem weight (g)} \times 0.5$$

where the value 0.5 was the mean efficiency of the squeezing machine, as calculated by (1 − bagasse weight)/stem weight.

Photosynthesis rates were measured in an upper fully expanded leaf taken from four plants per treatment at −1, 7, 15, 30, 45, and 60 DAT using a portable photosynthesis measurement system (LI-6400XT, LI-COR) equipped with a 2×3 cm^2 LED chamber (LI-6400-02B, LI-COR). Soil plant analysis development (SPAD) values were then measured on the same leaves with a chlorophyll meter (SPAD-502, Konica Minolta). All measurements were carried out between 10:00 am and 2:00 pm at a photosynthetic flux density (PFD) of 2,000 μmol m^{-2} s^{-1}, leaf temperatures of 25–30°C, a leaf to air vapor pressure difference of 1.5–3.5 kPa, and a CO_2 concentration of 400 µmol mol^{-1}.

The quality of the floodwater was checked with a dissolved oxygen meter (ID-150, Iijima) at the soil surface, a pH meter (B-71X, Horiba), and an electronic conductivity (EC) meter (B-771, Horiba). Oxygen levels gradually decreased until 10 DAT, after which they remained constant at around 0.8 mg L^{-1}. The pH did not change greatly, while EC increased at a constant rate to a level that was 2.5 times higher by the end of the treatment (Fig. 2.1). The total incident solar radiation in the glasshouse was approximately 1,280 MJ m^{-2}, with an average of 4.9 ± 2.1 MJ m^{-2} d^{-1}. The average daily maximum and minimum temperatures were 38.2 ± 5.8 and 22.7 ± 4.4ºC, respectively.

Results are given as means ± standard deviations. Mean values for each treatment were compared using a Fishers Least Significance Difference (LSD) test with a 5% level of significance.

2.4 Results

2.4.1 Growth of shoot and yield

Plants that experienced drought and prolonged drought exhibited a yellowing of the lower leaves, which proceeded to the upper leaves when drought conditions were extended. Eventually, some of the leaves died, resulting in the LA decreasing by 15.0% in drought conditions and 32.2% in prolonged drought conditions at 30 DAT. Furthermore, even re-irrigated plants had a lower LA than control plants during the experiment. The stem and total dry weight of plants that experienced drought and prolonged drought conditions were also lower than in the control plants, following a similar pattern to that observed for LA. By contrast, flooded plants, including flood, prolonged flood, and combination (F + PD and PF + PD) treatments, did not exhibit any change in leaf color, but LA did slightly decrease by 12.2%–14.4% at 30 DAT. Following flooding, the stem and total dry weight increased in a similar manner to control plants, except when prolonged flooding was followed by prolonged drought (PF + PD), which led to a reduction in stem weight (Fig. 2.2).

In the control plants, the sucrose content of the sugarcane juice started to increase from 15 DAT, following which the accumulation gradually accelerated (Fig. 2.3), demonstrating that the treatment occurred just before the period of accumulation began. Plants that were exposed to the first drought treatment, which was applied until 15 DAT, experienced a 14% increase in sucrose content. By contrast, the sucrose content was maintained at a low level or decreased as a result of prolonged drought until 30 DAT. The sucrose content was not affected by the flood treatments, and subjecting plants to prolonged drought after a flood (F + PD) may even have facilitated the accumulation of sucrose. The analysis of the concentration of various ions in the juice showed that sodium levels increased significantly in all flood and combination treatments (Fig. 2.4), and also remained high following re-irrigation. During flooding, sodium mostly accumulated in the bottom parts of the plant, whereas the upper parts retained the same low concentration as was seen in the other treatments (Fig. 2.5). At the time of harvest (75 DAT), sub-stem length, stem fresh weight, sucrose content, and sugar yield did not differ between treatments (Table 2.1).

2.4.2 Root growth and development

The root dry weight of plants decreased by 10% under drought conditions and 20% under prolonged drought conditions, and remained lower than the control even after re-irrigation (Fig. 2.6). A decrease in root dry weight was also observed in flooded plants, including the combination treatments (flood = 24.0%, prolonged flood = 25.3%, F + PD = 20.4%, and PF + PD = 25.3%) at 30 DAT, as a result of some of the roots rotting. The root growth in plants exposed to all of these treatments had recovered by 75 DAT, however, with no significant differences from the control plants.

Flooded plants produced three types of adventitious roots after flooding, an increased number of which were observed during prolonged flooding (Fig. 2.7). The first type of root appeared from the nodes under the water a few d after flooding and were initially white in color but then changed to pink. These roots were most developed in length and size at the top node, decreasing toward the bottom nodes. A second type of root then developed from the first type, which were numerous, small in size, thin, grew upward against gravity, and pink in color. Under prolonged flooding, a third type of root then emerged at the aerial nodes, commonly being found at the 1st–3rd nodes above the water. These roots were few in number, hard, and short (3–5 cm length), with a deep red color and slow growth. It was also found that the growth of adventitious roots increased under prolonged flooding, with the dry weight of roots being 80.2% higher under the prolonged flooding and PF + PD treatments than under the normal flooding and F + PD treatments, with significant differences at 30 DAT (Fig. 2.6). Aerenchyma developed in the cortex of these roots, increasing their porosity under prolonged flooding (Fig. 2.8). However, once the plants had been drained, these roots no longer grew, and they dried up.

2.4.3 CO_2 assimilation

Under drought conditions, CO_2 assimilation and transpiration deceased in accordance with the stomatal closure (Fig. 2.9). Plants that were exposed to drought and prolonged drought conditions had a 66% lower CO_2 assimilation rate than control plants at 15 DAT but then recovered following re-irrigation. By contrast, the flooding and prolonged flooding treatments only slightly affected the photosynthesis of the plants; and photosynthesis was unaffected when the soil was dehydrated after flooding (F + PD and PF + PD). Drought conditions reduced SPAD, and it was found that these effects may continue even after re-irrigation (Fig. 2.9). By contrast, SPAD was maintained at a fairly high level for all other treatments (flood, prolonged flood, F + PD, and PF + PD).

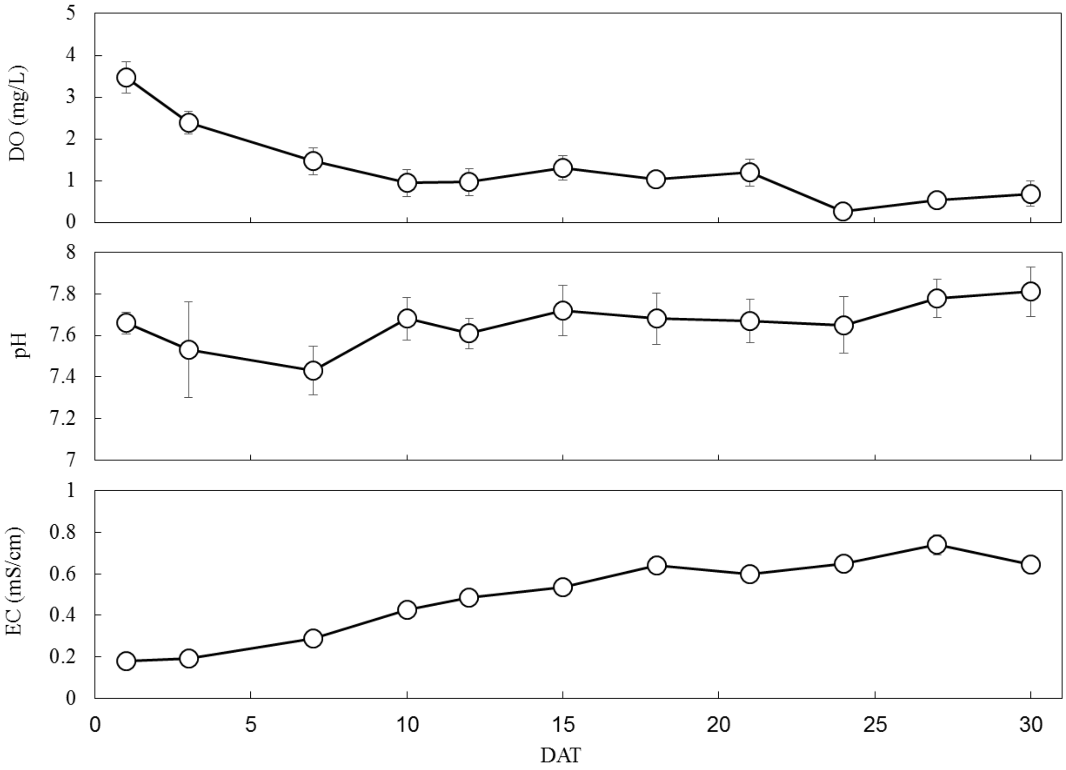

Fig. 2.1. Transition of water in flood treatments during 1-30 DAT included dissolved oxygen (DO) at soil surface or 35 cm water depth, pH and electronic conductivity (EC). Bar show means ± SD (n=10). DAT: days after treatment.

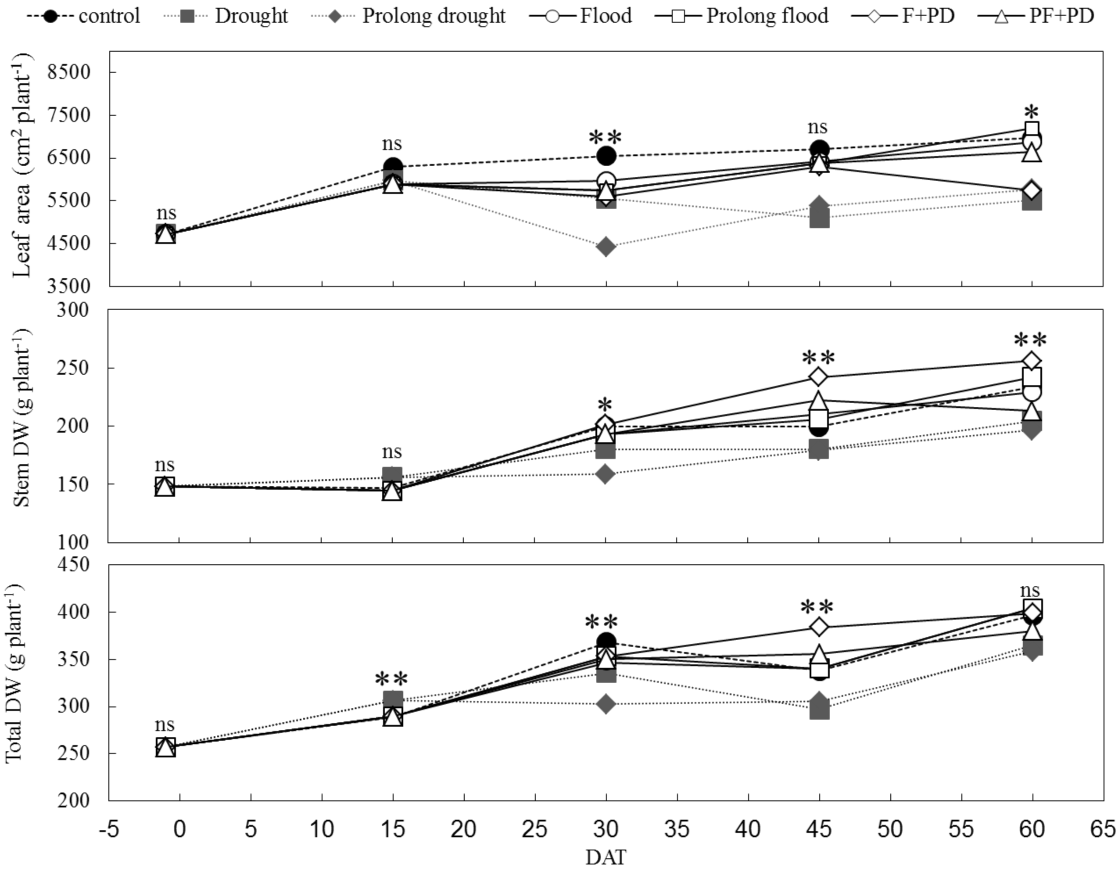

Fig. 2.2. Leaf area, stem DW (dry weight) and total DW of sugarcane cv. NiF8 compared with 7 treatments as control, drought (15 days), prolong drought (30 days), flood (15 days), prolong flood (30 days), F+PD (flood + prolong drought) and PF+PD (prolong flood + prolong drought) at sampling date: 1 day before treatment (-1), 15, 30, 45 and 60 DAT.

Note: *, ** indicate significant effect of treatments in each sampling at $P \leq 0.05$ and 0.01, respectively. ns indicate not significant (n=3). DAT: days after treatment.

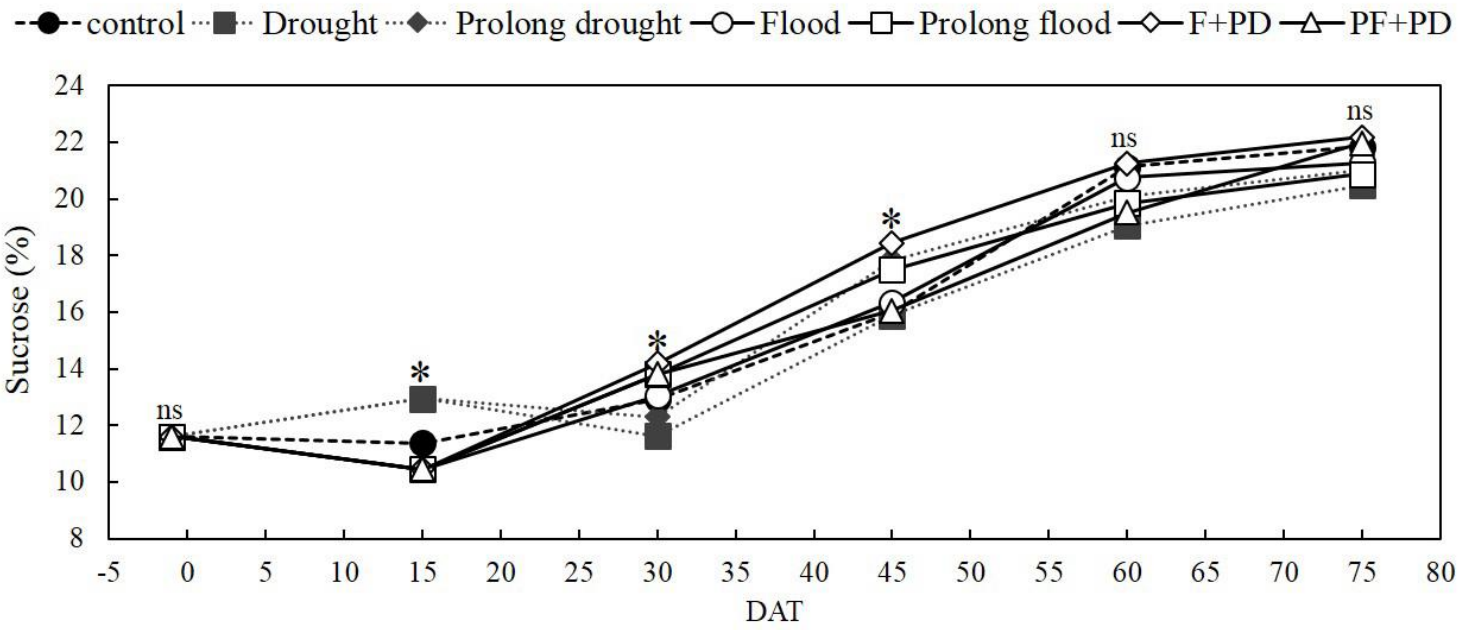

Fig. 2.3. Percentage of sucrose of sugarcane cv. NiF8 compared with 7 treatments as control, drought (15 days), prolong drought (30 days), flood (15 days), prolong flood (30 days), F+PD (flood + prolong drought) and PF+PD (prolong flood + prolong drought) at sampling date: 1 day before treatment (-1), 15, 30, 45, 60 and 75 DAT.

Note: *, ** indicate significant effect of treatments in each sampling at $P \leq 0.05$ and 0.01, respectively. ns indicate not significant (n=3). DAT: days after treatment.

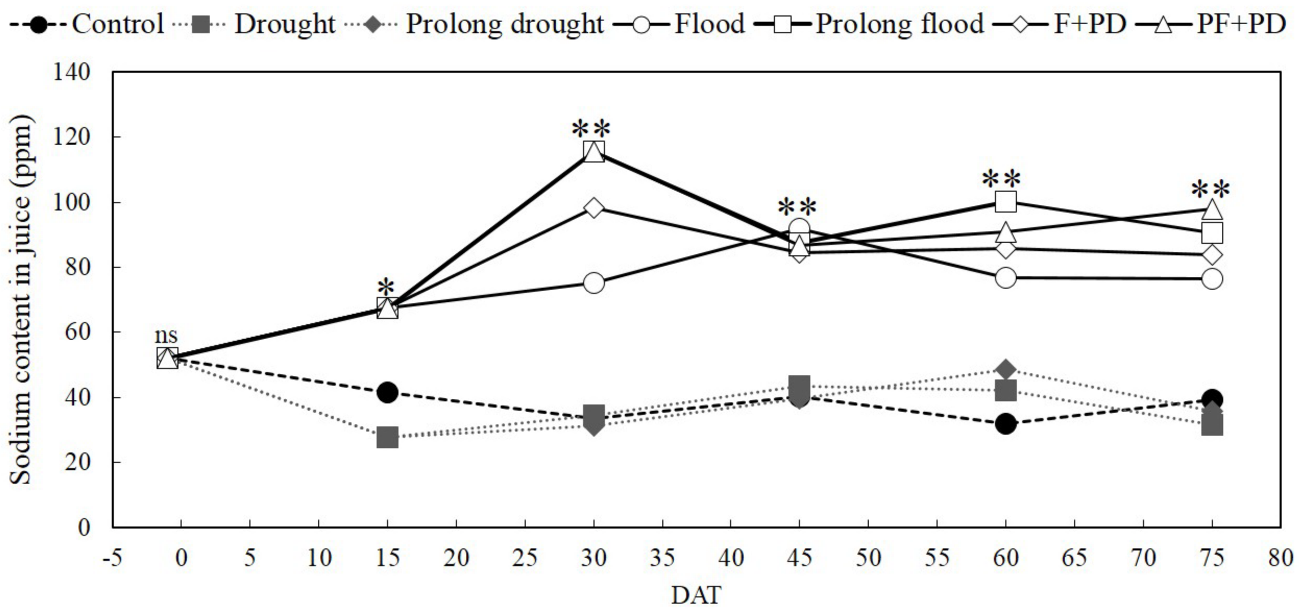

Fig. 2.4. Total sodium concentration in juice of sugarcane cv. NiF8 compared with 7 treatments as control, drought (15 days), prolong drought (30 days), flood (15 days), prolong flood (30 days), F+PD (flood + prolong drought) and PF+PD (prolong flood + prolong drought) at sampling date: 1 day before treatment (-1), 15, 30, 45, 60 and 75 DAT.

Note: *, ** indicate significant effect of treatments in each sampling at $P \leq 0.05$ and 0.01, respectively. ns indicate not significant (n=3). DAT: days after treatment.

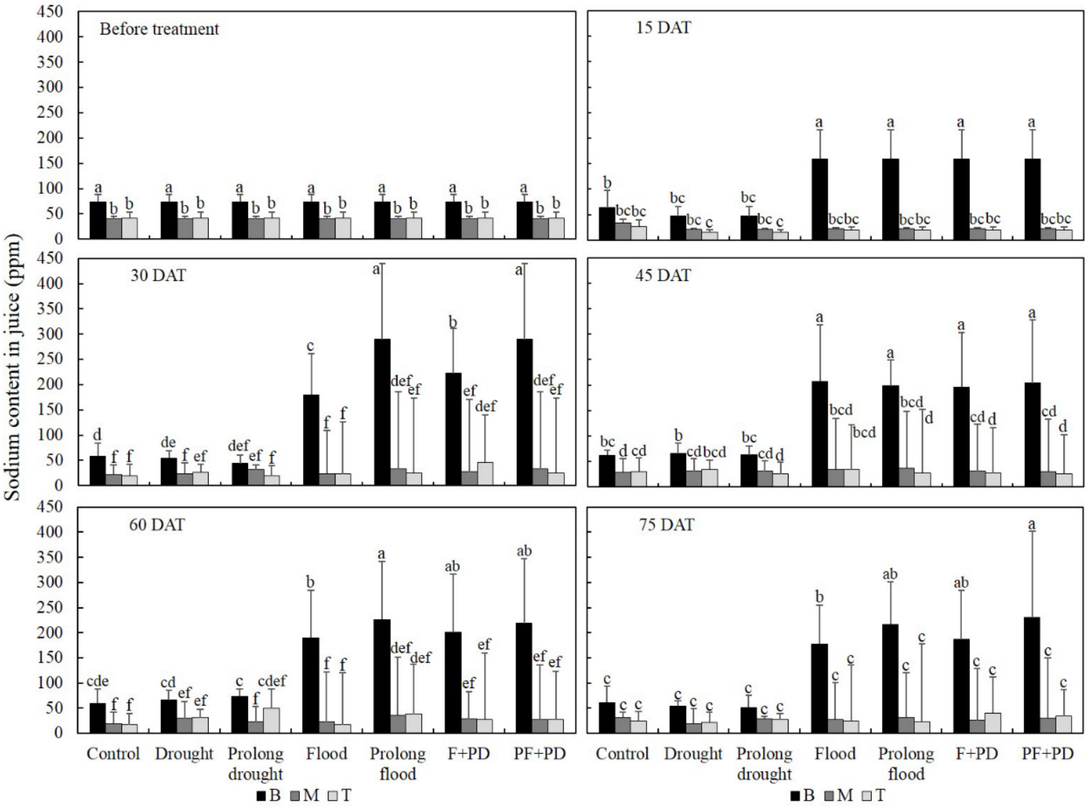

Fig. 2.5. Sodium concentration in juice separated by bottom (B), Middle (M) and top (T) of stem compared with 7 treatments as control, drought (15 days), prolong drought (30 days), flood (15 days), prolong flood (30 days), F+PD (flood + prolong drought) and PF+PD (prolong flood + prolong drought) at sampling date: 1 day before treatment, 15, 30, 45, 60 and 75 DAT.

Note: Mean with the same letter in each sampling indicates not statistically different (P ≤ 0.05) as determined by LSD multiple comparison test (n=3). Bar show means ± SD (n=10). DAT: days after treatment.

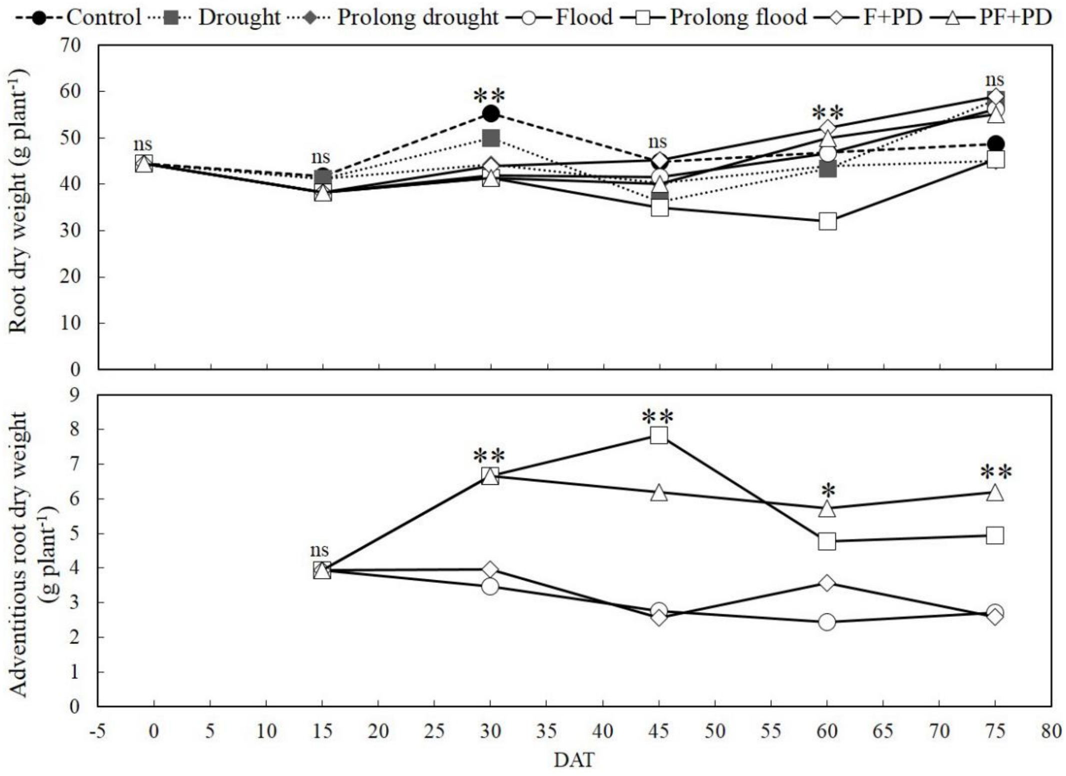

Fig. 2.6. Root dry weight and adventitious root dry weight of sugarcane cv. NiF8 compared with 7 treatments as control, drought (15 days), prolong drought (30 days), flood (15 days), prolong flood (30 days), F+PD (flood + prolong drought) and PF+PD (prolong flood + prolong drought) at sampling date: 1 day before treatment (-1), 15, 30, 45, 60 and 75 DAT of root dry weight and 15, 30, 45, 60 and 75 DAT of adventitious root dry weight.

Note: *, ** indicate significant effect of treatments in each sampling at $P \leq 0.05$ and 0.01, respectively. ns indicate not significant (n=3). DAT: days after treatment.

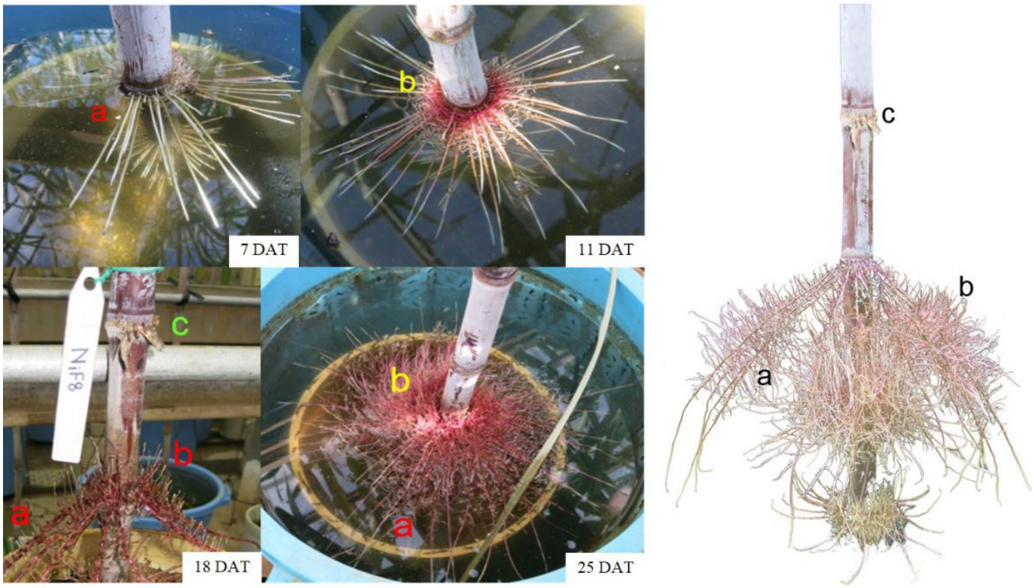

Fig. 2.7. Development of adventitious root at 7, 11, 18 and 25 days under flood condition. Three types of root were emerged, the letter a indicated the first type of roots appeared from the nodes under the water a few d after flooding and were initially white in color but then changed to pink. These roots were most developed in length and size at the top node, decreasing toward the bottom nodes. Afterwards, b type of roots were developed from a type with numerous, small in size, thin and grew upward against gravity, and pink in color. Under prolong flood c roots were third type of root then emerged at the aerial nodes, commonly being found at the 1st–3rd nodes above the water. These roots were few in number, hard, and short (3–5 cm length), with a deep red color and slow growth. DAT: days after treatment.

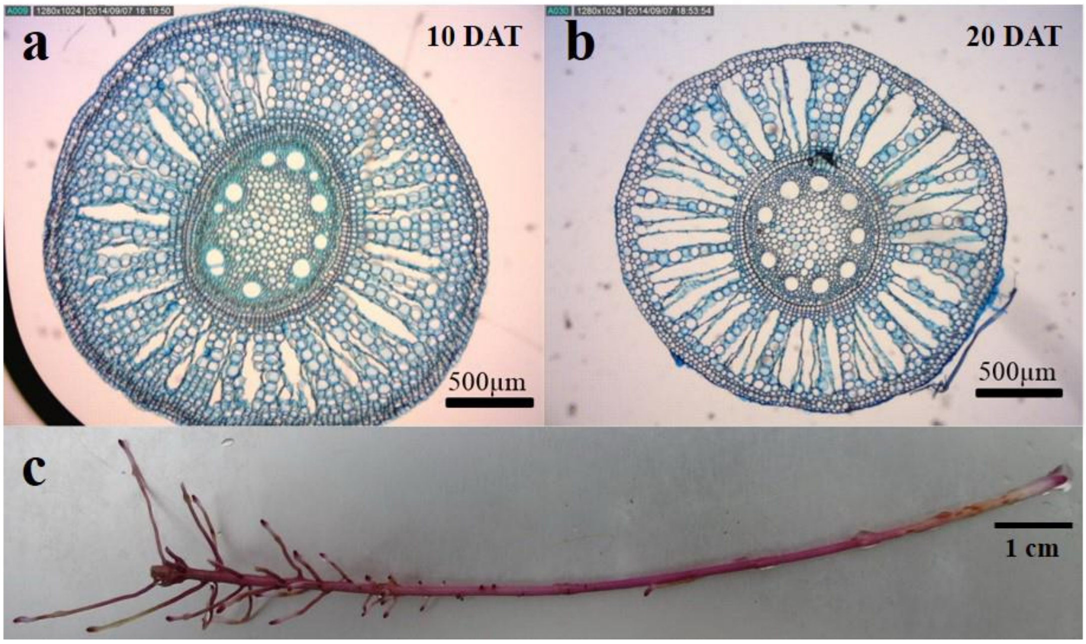

Fig. 2.8. Development of aerenchyma increased porosity in the cortex of adventitious during flood. Cross section were made at 1 cm above the tip at 10 and 20 DAT (pictures a, b and c). Picture c indicate adventitious root at 10 DAT.

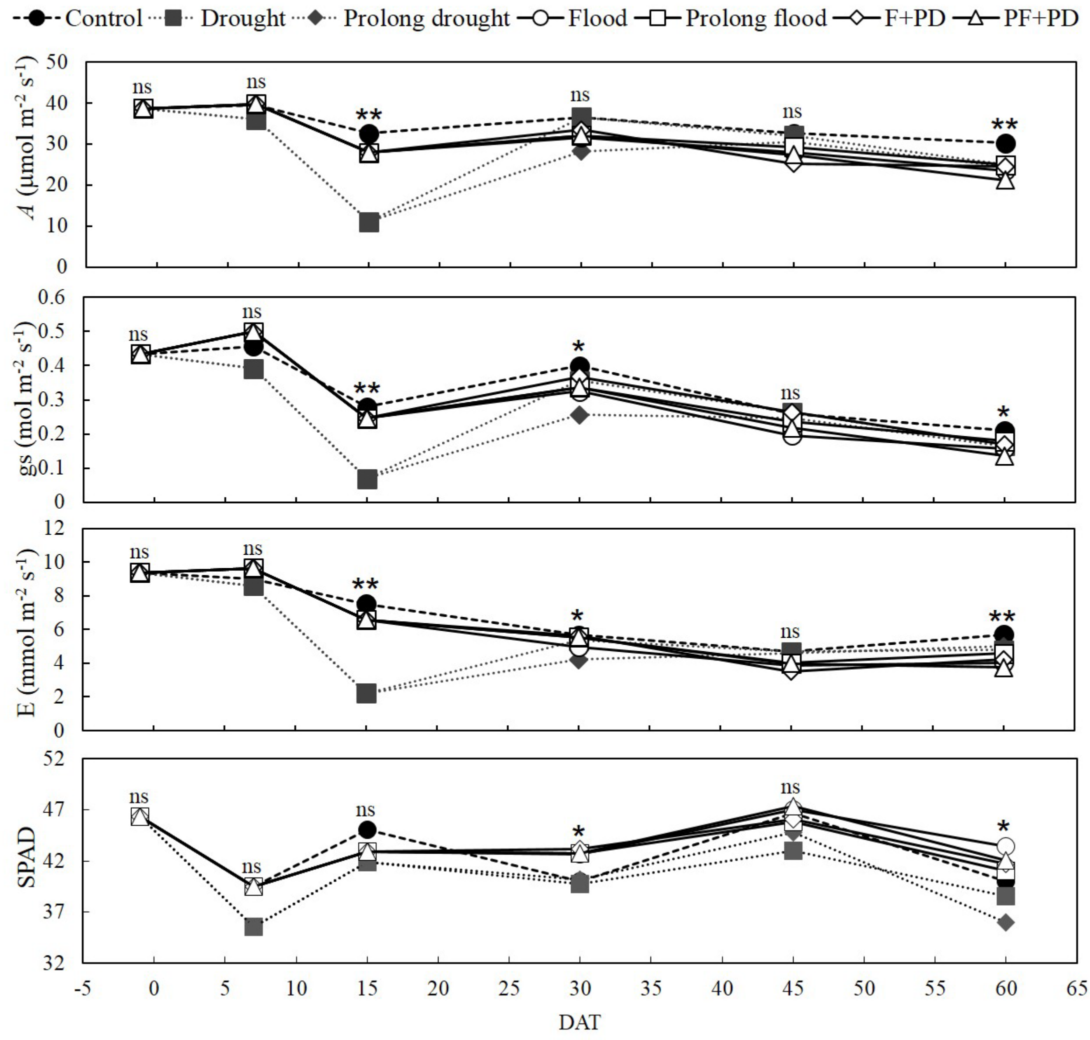

Fig. 2.9. CO_2 assimilation (A), stomatal conductance (gs), transpiration (E) and SPAD of sugarcane cv. NiF8 compared with 7 treatments as control, drought (15 day), prolong drought (30 days), flood (15 days), prolong flood (30 days), F+PD (flood + prolong drought) and PF+PD (prolong flood + prolong drought) measured at 1 day before treatment (-1), 15, 30, 45 and 60 DAT.

Note: *, ** indicate significant effect of treatments in each sampling at $P \leq 0.05$ and 0.01, respectively. ns indicate not significant (n=3). DAT: days after treatment.

Table 2.1. Table 1. Sub stem length, stem fresh weight, leaf area, total dry mass, percentage of sucrose, percentage of total sugar and sugar yield of sugarcane cv. NiF8 under drought, flood and combination treatment.

Treatment	Sub stem length (cm plant^{-1})	Stem fresh weight (g plant^{-1})	Leaf area (cm^2 plant^{-1})	Total dry mass (g plant^{-1})	Sucrose (%)	Total sugar (%)	Sugar yield (g stem^{-1})
Control	320.0a	1,515.7a	7,852.9a	450.5a	21.8a	22.2ab	165.6a
Drought	318.7a	1,368.7a	5,367.0d	395.9a	20.5a	21.0c	140.0a
Prolong drought	326.3a	1,464.7a	6,815.9ab	420.0a	21.0a	21.3bc	153.8a
Flood	316.0a	1,428.3a	6,164.4bcd	426.5a	21.3a	21.6abc	152.1a
Prolong flood	328.0a	1,566.3a	6,413.2bcd	430.8a	20.7a	21.3bc	163.7a
F+PD	318.7a	1,515.0a	6,649.7bc	459.5a	22.2a	22.7a	167.9a
PF+PD	311.3a	1,414.7a	5,683.1cd	434.0a	21.2a	22.3ab	155.3a

Treatments included control, drought (15 days), prolong drought (30 days), flood (15 days), prolong flood (30 days), F+PD (flood + prolong drought) and PF+PD (prolong flood + prolong drought) at harvest (75 DAT). DAT: days after treatment. Mean with the same letter in each column indicates not statistically different ($P \leq 0.05$) as determined by LSD multiple comparison test (n=3).

2.5 Discussion

CO_2 assimilation and the transpiration rate were dramatically reduced when plants were subjected to drought (Fig. 2.9) due to the stomata closing to prevent transpiration loss, which reduced the amount of CO_2 required for photosynthesis (Koonjah et al., 2006; Cornic and Massacci, 1996). The finding that stomatal conductance reduced alongside CO_2 assimilation matches the findings of a previous study on severe drought, which showed that stomatal conductance and water use efficiency declined by 5% and 7% of the control, respectively (Joseph and Leon, 2009). CO_2 assimilation, stomatal conductance, and transpiration rate in plants that were exposed to drought and prolonged drought treatments increased and recovered following re-irrigation, however (Fig. 2.9). By contrast, the flood and combination treatments slightly reduced CO_2 assimilation compared with the control. The CO_2 assimilation in plants that were exposed to flood and prolonged flood treatments were no different from the control plants at 30 DAT, and plants that were exposed to the treatments that combined flooding with prolonged drought may use the moisture remaining in the drained soil together with the low level of irrigation water to maintain CO_2 assimilation until re-irrigation. These results are consistent with a previous study that reported the positive response of sugarcane gas exchange rates to periodic 7 days flooding (Glaz et al., 2004) and another study which reported that periodic flooding every months did not affect CO_2 assimilation of Ho 01-12 (energy cane), HoCP 96-540 (sugarcane), or L99-226 (sugarcane), with only L79-1002 (energy cane) experiencing a 50% reduction in cane plants and a 48% reduction in ratoon cane (Viator et al., 2012). However, in the combination treatments (F + PD and PF + PD), CO_2 assimilation was reduced during prolonged drought but then increased when the plants were re-irrigated. In the early stages of flooding (7 DAT), stomatal conductance and transpiration increased to higher levels than control plants. However, when the flooding was extended, stomatal conductance and transpiration reduced in the same way as CO_2 assimilation and were lower than the control (Fig. 2.9). This matches a previous study that showed that a 41 days flood reduced CO_2 assimilation, while stomatal conductance was higher than control plants (Hidaka and Karim, 2007).

Plants that were exposed to drought and prolonged drought experienced a reduction in growth. This matches the findings of a previous study, which demonstrated that water deficit resulted in a reduction in turgor pressure, the interruption of water flow from the xylem to the surrounding elongation cells, and a slowing down of the growth process, particularly in terms of a decrease in cell elongation and cell volume, an increased concentration of cell sap, and the progressive dehydration of the protoplasm (Larcher, 2001; Nonami, 1998). Low biomass accumulation under water stress has been attributed to a reduction in light interception, plant extension rate, and photosynthesis (Koonjah et al., 2006). However, following re-irrigation, the drought plants exhibited slightly increased LAs, stem dry weights, and total dry weights, although these were still lower than the control plants. By contrast, the flood and combination treatments resulted in plants initially having a slightly reduced growth rate than control plants and then a slightly higher growth rate than the control plants, even under prolonged flooding. This is consistent with the previous finding that the total dry weight of the roots, leaves, and stalks of flood plants was 16% higher than control plants (Hidaka and Karim, 2007). The F + PD and PF + PD treatments resulted in plants having slightly reduced growth, but this then increased when the plants were re-irrigated (Fig. 2.2). At the time of harvest, the sub-stem length, stem fresh weight, and total dry weight had recovered for all treatments, while LA was still affected and different between the treatments (Table 2.1).

Prolonged drought reduced the root dry weight by 19.9% at 30 DAT, which matches the previous finding that the hypocotyl length, and the fresh and dry masses of shoots and roots of alfalfa (Medicago sativa) decreased under water deficit (Zeid and Shedeed, 2006). Similarly, prolonged flooding and the PF + PD treatment reduced the dry root weight by 25.3% at 30 DAT (Fig. 2.6). These results are consistent with a previous study on roots under flooded conditions, which demonstrated that the root hairs died and the original roots became blackened and rotten, leading to the arrest of root respiration and affecting important metabolic activities of the plants (Gomathi et al., 2014). However, the plants compensated for this by producing adventitious roots that emerged from the root primordia at nodes under the water and from aerial nodes, with increased numbers being seen when prolonged flooding had occurred (Fig. 2.6). These roots developed as a result of the hormonal imbalance that is induced by hypoxia due to the low oxygen supply to the submerged tissue and were located in the upper layer of water, which has a higher oxygen content (Gomathi et al., 2014). Three types of roots were produced after flooding: the first type emerged from the nodes that were located under the water, the second type developed from these first roots and grew upward against gravity, and the third type emerged from aerial nodes above the water (Hidaka and Karim, 2007). As a result of this growth, the root dry weight was 41.0% higher under prolonged flooding compared with normal flooding (Fig. 2.6).

These roots also exhibited an increased porosity as a result of aerenchyma developing in the cortex (Fig. 2.8). It has previously been reported that flooding for 3 months led to a 4–15 times increase in root development, a 108% greater aerenchyma pipe extension, and a 115% greater aerenchyma pipe diameter (Gilbert et al., 2007), while flooding for 120 days to a level 30 cm above the top of the soil led to sugarcane clone I 6-04 having the highest root dry weight at 28.3 g/plant (Begum et al., 2013). During floods, plants produce roots and exhibit ethylene-dependent death and lysis, which lead to the formation of continuous gas-filled channels (aerenchyma) that help the plants to maintain their root activity and supply the necessary oxygen (Drew, 1997). Thus, the numerous roots that grow during floods are better adapted to these conditions than the original roots, containing well-developed aerenchyma (Laan et al., 1991). Since root elongation was closely related to the oxygen concentration in the root zone, the internal aeration of the plants may have been achieved by increasing the root porosity and developing aerenchyma, which may have helped the plants to continue to take up water and nutrients, and may have compensated any losses associated with flooding (Begum et al., 2013; Gomathi et al., 2014; Gilbert et al., 2007). However, following drainage, it was found that some of the original roots were damaged, and the adventitious roots dried out and became non-functional. Thus, the flooded plants required time to develop new roots to support and recover their growth.

The sucrose content of the sugarcane juice increased during the early stages of drought but then decreased under prolonged drought conditions when the plants were placed under extreme stress. These findings support those of a previous study that showed that sucrose content increased during a period of low rainfall or under dryland conditions but was reduced under extreme drought conditions (Robertson et.al., 1999a); and similarly, a 6 weeks drought during the grand growth phase was found to reduce the sucrose content by an average of 4.7% and sugar yield by 11.7%–19.1%

(Wiedenfeld, 2000). In the present study, however, sucrose content increased again following re-irrigation. In plants that were exposed to the flood and combination treatments, the sucrose content decreased in the early stages of flooding and then dramatically increased to reach levels that were similar to the control plants, even during prolonged flooding or where flooding was followed by drought (Fig. 2.3). These results are consistent with the previous finding that sugar yield was not affected in cultivar CP 72-1210 when grown with a water table depth of 45 or 75 cm (Pitts et al., 1990), while 2 days periodic floods in each of eight 14 days cycles per year increased the cane and sucrose yield in cultivars CP 72-2086 and CP 80-1827 (Glaz and Gilbert, 2006). However, in the present study, all treatments had an increased sucrose content once the treatments had finished.

During flooding, the concentrations of various ions, such as $NH4^+$, K^+, Mg^+, Cl^-, $PO4^-$, and $SO4^-$, were reduced in the sugarcane juice and remained at a lower level than the control even once the water had been drained and the conditions had returned to normal. Both Ca^+ and F^- also increased during flooding but then returned to similar levels to the control following drainage (data not show). By contrast, the concentration of sodium (Na^+) was higher in the flooded plants than in the control and drought plants. Furthermore, during prolonged flooding, the sodium content of plants was 245.5% higher than in the control plants, representing a 71.3% increase as a result of flooding, which was maintained even after the water had been drained or the plants had been placed under prolonged drought conditions (Fig. 2.4). The highest sodium content was found in the bottom part of the stem, and it was here that the levels increased during prolonged flooding, while the middle and top parts of the stem had similar sodium concentrations as the control plants (Fig. 2.5). However, there is still a lack of information about this increase in sodium content in the juice of flooded sugarcane plants, and so its role in flood tolerance remains unclear. Thus, further study on the relationship between sodium accumulation and physiological changes under flood conditions may help to explain this phenomenon in the future.

2.6 Conclusion

Our study showed that CO_2 assimilation was reduced dramatically in sugarcane plants that were exposed to drought and prolonged drought treatments but recovered once the plants were re-irrigated. By contrast, the flood and combination treatments did not affect CO_2 assimilation, even when the water was drained or the plants were subsequently exposed to prolonged drought. During flooding, adventitious roots with well-developed aerenchyma were produced, the number of which increased under prolonged flooding, which may help plants to offset the losses associated with flooding. The expansion of leaf area was interrupted by drought and remained at a low level even after re-irrigation, while this increased once the flood and combination treatments had finished. The stem and total dry weight increased as a result of the flood and combination treatments but reduced during the drought treatments. At the time of harvest, there were no significant differences in stem fresh weight, sucrose content, or sugar yield between treatments. Flooding induced the accumulation of sodium in sugarcane juice at the bottom of stem, with a particularly high accumulation following prolonged flooding, and this remained even after the soil water had been drained and re-irrigated. Therefore, it is concluded that the formation of adventitious roots may offset the negative effects of root death and help plants to maintain their growth and yield of sugarcane during a flood.

CHAPTER 3

Changes in Photosynthesis, Growth, and Sugar Accumulation of Commercial Sugarcane Cultivars and *Erianthus* under Flood Conditions

3.1 Abstract

Sugarcane (*Saccharum* spp.) is an economical crop in the tropical and subtropical countries. However, because of global climate change, flooding has become problematic, particularly during the rainy season, in Thailand. We investigated the effects of floods on three commercial sugarcane cultivars, namely NiF8, U-thong 6 (UT6), and U-thong 9 (UT9), as well as *Erianthus* spp. Growth was assessed using a pot experiment in a glasshouse with two treatments: 1) control and 2) 60 days of flooding followed by 30 days of normal conditions. In comparison with control, during prolonged flooding, *Erianthus* showed greatly decreased CO_2 assimilation, whereas NiF8, UT6, and UT9 showed slightly declined CO_2 assimilation. Growth in plants subjected to 60 days of flooding was less influenced by floods while sucrose content was not affected except in UT6. During flooding, some roots died, resulting in plants compensating adventitious roots to offset the negative effects of root death and to assist them maintaining their growth, which appeared from the submerged nodes, with different characteristics for each cultivar. However, 30 days after draining, roots remained damaged, while adventitious roots died, resulting in a lesser growth as compared with the control, but it did not significantly affect

sucrose content and sugar yield. This study suggests that sugarcane plants need to produce the adventitious root to compensate their roots death during flooding and require time to recover their root system after flooding for obtaining the optimum yield and quality at harvest.

3.2 Introduction

Sugarcane (*Saccharum* spp.) is an economically important crop for sugar and bioenergy production in many tropical and subtropical countries. High sucrose content in cane is one of priorities for sugarcane farmers and sugar mills to obtain high price and sugar yield, respectively. Moreover, the residues or by-products of sugar mills, biomass and bioethanol, can be utilized as an alternative source of energy. In Thailand, sugarcane production has dramatically increased as a result of an encouragement by the government for switching arable lands from rice to sugarcane for better returns (USDA Foreign Agriculture Service, 2014). However, because of the rising global temperature accompanied with changes in weather and climate, which results in more frequent and more severe flooding, negative effects on sugarcane are often observed, particularly during the rainy season. Furthermore, the change from paddy fields to sugarcane fields may increase the risk of floods and cause problems for cane growers especially in the central basin. Two predominant sugarcane planting patterns exist: 1) at the end of the rainy season (October–January) in irrigated areas and 2) in the early part of the rainy season (April–May) in rain-fed areas. The rainy season is from May to September and there are two rainfall peaks in May and August (Chokngamwong & Chiu, 2006). During the rainy season, sugarcane planted during the early part of the rainy season is 5–6 months of age, coinciding with the grand growth phase (4–10 months), which is important for cane formation, elongation, and accumulation of yield (Gascho, 1985). Thus, flooding at this stage may adversely affect growth and eventually cause negative effects on yield and quality of sugarcane during the maturation or ripening phase.

Generally, the extent of damage by flooding on sugarcane differs between the genotypes, environmental condition, stage of growth, and duration of stress (Gomathi et al., 2014). Cane and sugar yield were decreased because of decrease in photosynthesis, root development, leaf area (LA), LA index (LAI), tiller production, stalk height, and sucrose yield (*Gomathi et al., 2014*; Viator et al., 2012; Webster & Eavis, 1972). However, previous study reported that flooding for 15 days to 30 days when sugarcane was 6 months old did not affect CO_2 assimilation, sucrose content, stem and total dry weight while LA was reduced (Jaiphong et al., 2016). Sugarcane growth has been shown to be restricted via decrease in metabolic activity of roots because of hypoxia occurring during flood conditions (Gomathi et al., 2014). Plants adapt to flooding by developing adventitious roots with well-developed aerenchyma to assist the maintenance of root activity and the supply of required oxygen, and also contribute to higher dry matter accumulation (Drew, 1997; Gilbert et al., 2007; Gomathi et al., 2014; Jaiphong et al., 2016).

Recently, the genus *Erianthus*, one of the *Saccharum* complex, has become important as a genetic resource in sugarcane breeding for biomass production because of its tolerance to extreme conditions, such as drought and flooding. A previous study identified root development of *Erianthus* in saturated soil as deep as 250 cm, whereas the root of Napier grass was found at <135 cm from the ground. Meanwhile, the pot experiment reported *Erianthus* increased biomass production and had well-developed aerenchyma on the roots of plants grown under waterlogged conditions (Matsuo et al., 2001). Moreover, inter-generic and inter-specific *Saccharum* hybrids of commercial sugarcane with *Erianthus arundinaceus* and *S. spontaneum* grew well under a flooded condition for 6 months (Deren et al., 1991). Thus, *Erianthus* is a potential genetic resource in sugarcane breeding not only for higher biomass production and drought tolerance but also for flood tolerance.

To the best of our knowledge, information regarding physiological characteristics and growth ability of commercial cultivars and *Erianthus* under a flooded condition is lacking. The objective of the present study was to determine changes in photosynthesis, growth, and sugar content in three commercial sugarcane cultivars and *Erianthus* under long-term flood conditions and after the change to normal conditions. Moreover, morphological changes and development of adventitious roots were analyzed for gaining a better understanding of its adaptive significance of tolerance.

3.3 Materials and methods

3.3.1 Study site, plant material and treatment

A pot experiment was conducted in a glasshouse at the University of the Ryukyus, Okinawa, Japan (26° 15′ N, 127° 45′ E; altitude 127 m) from May, 2014 to Feb, 2015. Three sugarcane cultivars, NiF8, U-thong 6 (UT6) and U-thong 9 (UT9), and *Erianthus* spp (*Erianthus*) were used. NiF8 is the most popular and commercial cultivar in Japan and has been reported to have flood tolerance because of less affected growth and well-developed adventitious roots to compensate for original roots during flooding (Hidaka & Karim, 2007; Jaiphong et al., 2016). *Erianthus* is one of the genus in Saccharum complex, adapting well to the environment, and an important genetic resource for sugarcane. Both were received from Tropical Agriculture Research Front, Japan International Research Center for Agricultural Science (JIRCAS) at Ishigaki, Okinawa. The other two sugarcane cultivars, UT6 and UT9, were introduced from Suphan Buri Farm Crops Research Center, Department of Agriculture, Thailand. Pest-checking was performed at the plant protection station Ministry of Agriculture, Forestry and Fisheries of Japan in Okinawa, 1 year before being used for the experiment. UT6 and UT9 were commercial cultivars, but there is no report of their flooding tolerance, though they are recommended to be planted in a central region of Thailand where there is a possibility of flooding every year.

The experiment simulated the planting pattern in Thailand. Matured stems of sugarcane and *Erianthus* were selected for making seedling, afterward fertile nodes were cut then germinated in a tray on 15 May 2014. One month after, seedlings were transplanted into 1/2000 a Wagner pots (a: are unit, pot diameter is 25 cm with 500 cm^2 surface area) filled with dark red soil (Shimajiri mahji), sand, and peat moss (1:1:1, v/v). Initially, an automatic drip irrigation system was used to water the plants for 15 min (495 mL) every morning. The level of irrigation was then doubled 3 months after transplanting by watering the plants at noon as well, and tripled 5 months after transplanting through an additional watering at evening. Through the experiment, tillers of sugarcane cultivars were immediately removed after emergence to avoid the competition within a plant and to understand better the treatment effects by focusing on the main stems, except *Erianthus* which has a great number of tillers.

Flooding treatment began during the rainy season (May–September) when the sugarcane plants were 4–6 months of age, i.e., during the grand growth phase. Six months after transplanting (November 21), uniform plants were selected and used for the experiment. Two treatments consisting of the control (C) and flooding (F) were established. Control plants were irrigated with an equivalent amount of 50% (v/v) of soil moisture, whereas during the flooding treatment, plants were submerged up to 35 cm above the soil surface in 45 L plastic buckets for 60 days. Subsequently, water was drained and plants were irrigated to the same extent as the control for 30 days.

Eighteen plants were prepared for each treatment. Plants were weekly fertilized by replacing irrigation with 500 mL of Hoagland's nutrient solution composed of 4 mM KNO_3, 6 mM $Ca(NO_3)_2 \cdot 4H_2O$, 2 mM $MgSO_4 \cdot 7H_2O$, 2 mM KH_2PO_4, 0.5 µM $CuSO_4 \cdot 5H_2O$, 6.3 µM $MnSO_4 \cdot 5H_2O$, 2 µM $ZnSO_4 \cdot 7H_2O$, 25 µM H_3BO_3, 0.3 µM $Na_2MoO_4 \cdot 2H_2O$, and 0.1 mM Fe(III)-ethylenediaminetetraacetic acid. During flooding treatment, the solution was not added into the flooding water but re-applied after draining. Plants were arranged with 40 × 90 cm spacing between plant and row in a completely randomized design.

3.3.2 Growth and sugar content evaluation

Three plants of each treatment were sampled on 1 days before the treatment started (–1), 60 days and 90 days after the treatment started (DAT) to examine plant growth, damage by flooding, plant adaptation and sucrose content. LA of the entire plant was measured using a leaf area meter (LI-3100, LI-COR). For stem sampling, stems were cut from the base, and stem length, the distance from the soil surface to the node of the fifth leaf below the top visible dewlap leaf, and stem weight were measured. Each stem was evenly separated into bottom, middle, and top by stem length and squeezed using a three-roller mill to obtain sugarcane juice from each part. For root sampling, adventitious roots appearing from the submerged stem were separately collected and dried in an oven at 80 ºC for 48 hours to determine their dry weights. After cutting the stem, underground roots in the pots were washed with water to separate soil, and once roots were dry, their dry weights were determined in the same way as adventitious roots. Underground root dry weight was determined for a single plant in each sugarcane cultivar, whereas *Erianthus* root included the tiller roots because it was difficult to separate roots of main stem from those of tillers. Therefore, root weight and total dry weight per plant of *Erianthus* was not compared with the three sugarcane cultivars. All parts of the sugarcane cultivars including leaf, dead leaf, leaf sheath, shoot (the upper part of stem), stem, root and adventitious root were dried in an oven at 80 ºC for 48 hour to determine their total dry weight. The juice was diluted 50 times with distilled water and filtered with a 0.45 μm membrane filter (ADVANTEC), following which the sugar content was analyzed by high-performance liquid chromatography system (LC-20A, Shimadzu) using a column SCR-101N with oven temperature of 50 ºC, flow rate of 0.8 ml/min and degassed extra pure water as the mobile phase. Sugar yield was roughly calculated using the following equation:

$$\text{Sugar yield} = \text{sucrose content (\%)} / 100 \times \text{stem weight (g)} \times 0.5$$

where the value 0.5 represents the mean efficiency of the squeezing machine, as calculated by (1 − bagasse weight) / stem weight.

3.3.3 Photosynthesis measurement

Photosynthesis rates were measured in third upper fully expanded leaves taken from three plants per treatment on –1, 9, 22, 30, 45, 56, 78, and 90 DAT using a portable photosynthesis measurement system (LCpro-SD, ADC) equipped with an LED chamber (5.8 cm^2). The soil plant analysis development (SPAD) values were then measured on the same leaf using a chlorophyll meter (SPAD-502, Konica Minolta). All measurements were conducted between 10:00 am to 02:00 pm at a photosynthetic flux density of 2,000 μmol m^{-2} s^{-1}, leaf temperatures of 25–30 °C, leaf to air vapor pressure difference of 1.5–3.5 kPa, and ambient CO_2 concentration.

3.3.4 Transition of water in flooding treatments

The O_2 concentration of the floodwater was assessed with a dissolved oxygen meter (ID-150, Iijima) using a plastic pipe with holes set around the root zone. The pipes were covered by the aluminum net and set vertically by filling the soil into the pot with a pH meter (B-71X, Horiba) and an electronic conductivity (EC) meter (B-771, Horiba). The oxygen level gradually decreased until 20 DAT, after which it remained constant at < 1.0 mg L-1. The pH did not change significantly in plant pots but increased in the water in the plastic buckets (check 1) and in water in the plastic buckets with pot soil (check 2). EC increased consistently in all pots, particularly in *Erianthus* pots, whereas check 1 was stable (Fig. 3.1). The averages of daily maximum and minimum temperatures were 35.0 ± 4.4 and 11.0 ± 2.8 ºC, respectively with an average relative humidity 69.27% ± 11.5%.

3.3.5 Statistic

Results are given as means ± standard deviations. Mean values between the control and flooding treatments were compared using t-test.

3.4 Results

3.4.1 CO_2 assimilation

At an early stage of flooding on 9 DAT, CO_2 assimilation (*A*) was not significantly different between the control and flooded plants in any sugarcane cultivars and *Erianthus*. Subsequently, *A* gradually decreased in accordance with the stomatal closure and transpiration in all cultivars (Figs. 3.2, 3.3, 3.4). Flooded plants of *Erianthus* showed a clear decrease in *A* when flooding was prolonged: *A* decreased to 3.7–4 μmol m^{-2} s^{-1} on 45 and 56 DAT, respectively, while those of NiF8, UT6, and UT9 were decreased to 10.7, 23.6, and 21.6 μmol m^{-2} s^{-1}, respectively on 56 DAT. After draining, all plants showed an increase in *A* but remained lower than the control even 30 d after draining (Fig. 3.2). Flooding decreased the SPAD value only in NiF8 though those in the other cultivars were unaffected (Fig. 3.5).

3.4.2 Growth of shoot and yield

On 60 DAT, the LA data of *Erianthus* was not collected due to low LA in the control plants because of a broken irrigation tube located in the control plants, resulting in wilting leaves. LA of NiF8, UT6, and UT9 did not significantly but decrease in comparison with the control and flooded plants. Some parameters related to plant growth were decreased by flooding but no significance except a decrease in leaf dry weight of UT6, number of nodes of UT9, and stem diameter of NiF8.

Flooding did not even affect stem weight significantly but reduced those of *Erianthus*, NiF8 and UT9, whereas that of UT6 slightly increased. After flooding, the total dry weight hardly changed (Table 3.1).

Similarly to the sampling 60 DAT, most of growth parameters were lowered by flooding. LA and leaf dry weight of all the sugarcane cultivars and *Erianthus* in the flooding treatment remained lower than the control on 90 DAT or 30 days after draining and especially those of UT6 significantly decreased by about 20%, respectively. Stem weight of *Erianthus* in the flooding treatment was slightly higher than that of the control, whereas those of the sugarcane cultivars were consistently lower. The total dry weight also did not significantly differ between control and flooding (Table 3.2).

3.4.3 Root and adventitious root growth

Root dry weight of UT6 and *Erianthus* significantly decreased in comparison with the control on 60 DAT, whereas those of UT9 and NiF8 decreased but with no significance probably because of some of the roots rotting under a flooding condition (Table 3.1). On 90 DAT, root dry weight remained affected by the flooding. The root dry weight of UT6, *Erianthus*, and NiF8 significantly decreased, whereas that of UT9 did not significantly decrease (Table 3.2).

Adventitious roots that emerged from root primordia at the nodes under water and aerial nodes were found a few days after flooding in all sugarcane cultivars and *Erianthus*. On 60 DAT, UT6 had the highest dry weight (6.3 g stalk^{-1}) (Table 1). The characteristics and quantity of emerged roots were dependent on the cultivar. *Erianthus* only produced a few roots per node irrespective of node position. NiF8, UT6, and UT9 showed the highest dry weights at node number 5, 4, and 3 above the ground, respectively, and gradually showed a decrease in submerged nodes according to depth and the nodes near the water level (Figs. 3.6, 3.7). After draining, these roots no longer grew, and subsequently dried up.

3.4.4 Sugar content and sugar yield

Among the sugarcane cultivars, NiF8 had the highest sucrose content and sugar yield on both 60 and 90 DAT, followed by UT6 and UT9, whereas *Erianthus* contained almost no sugar due to the low stem weight and sucrose content. On 60 DAT, sucrose content was negatively affected by flooding in UT6, but no significant difference between control and flooding treatments was confirmed in NiF8 and UT9. By contrast, flooding gave a positive effect on sucrose content of *Erianthus*. (Table 3.3). Analysis of sugarcane juice separately collected from three parts revealed that sucrose content of UT6 was significantly reduced by flooding at the bottom and middle part of stem. Sucrose content of NiF8 and UT9 in flooding was not significantly different from the control plants (Fig. 3.8). After draining and re-irrigation for 30 days, no significant difference in sucrose content was found (Table 3.3).

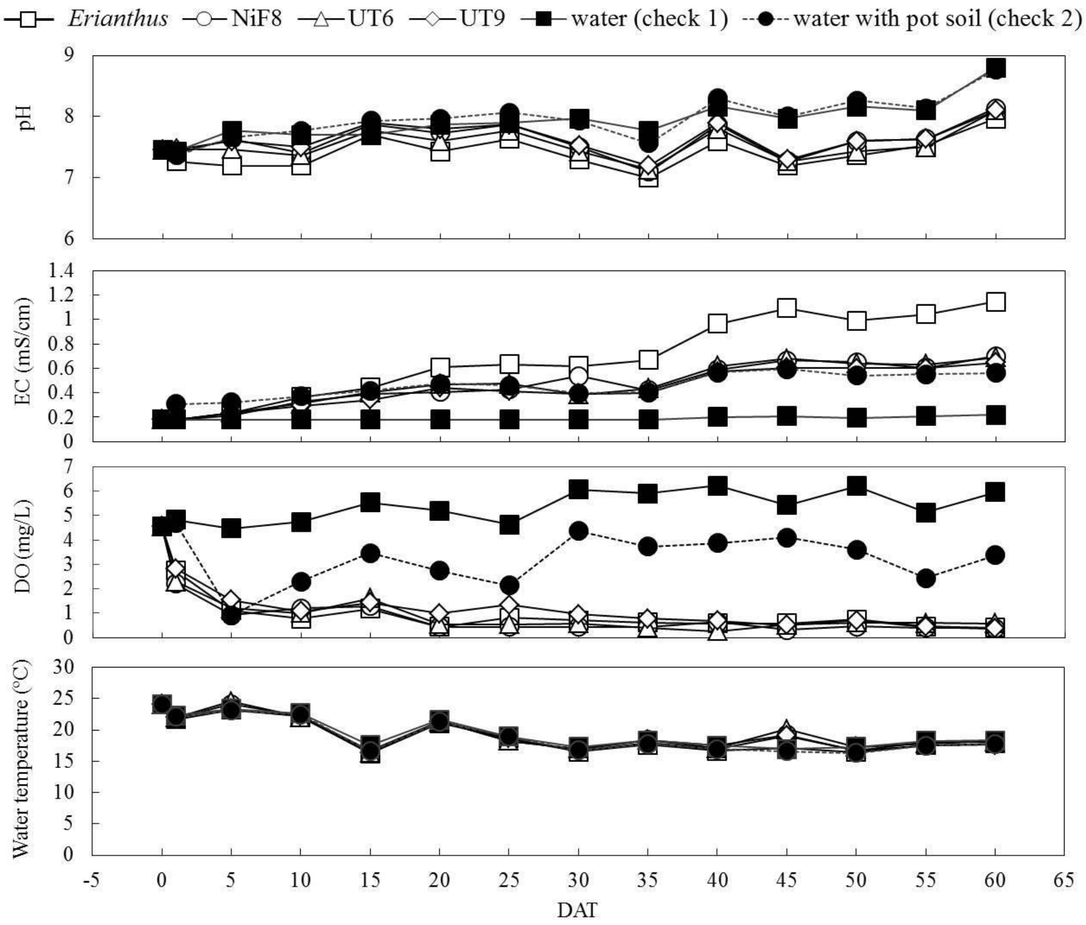

Fig. 3.1 Changes in pH, EC, DO and temperature of water in the bucket of flooded plants as *Erianthus*, NiF8, UT6, UT9, water in the plastic buckets (check 1) and water in the plastic buckets with pot soil (check 2) in the flooding treatment during 1–60 DAT (n=5).

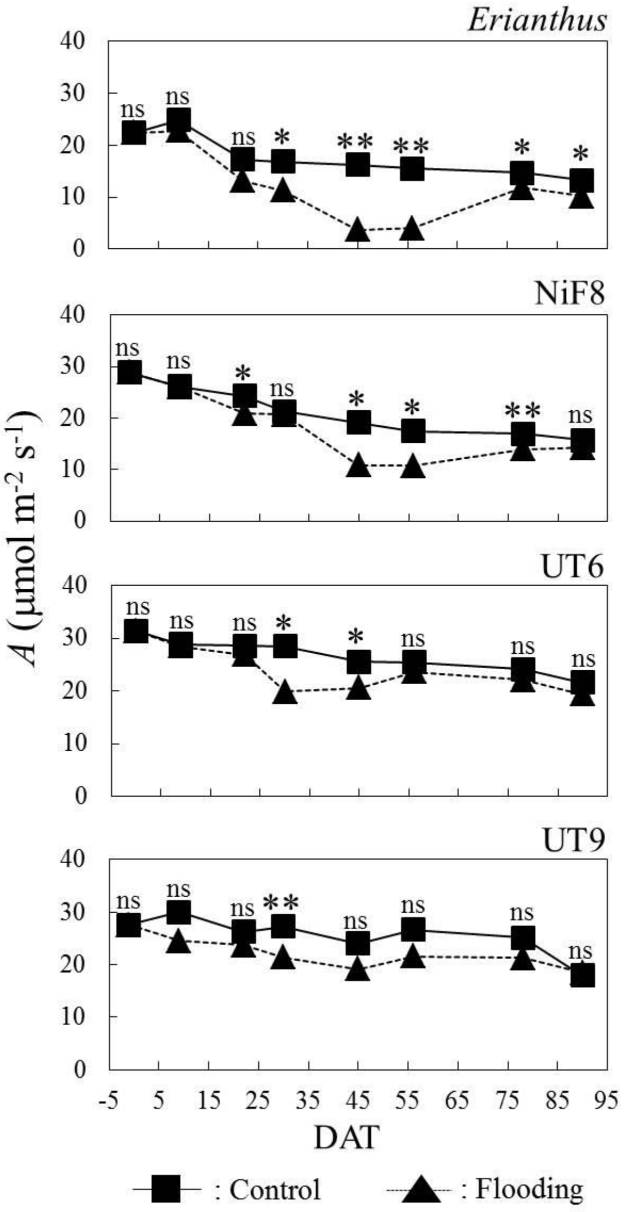

Fig. 3.2 Changes in A during flooding (1–60 days) and after draining (61–90 days).

Note: * and ** indicate significant differences between the control and flooded plants at $P < 0.05$ and 0.01, respectively and ns not significant (n=3).

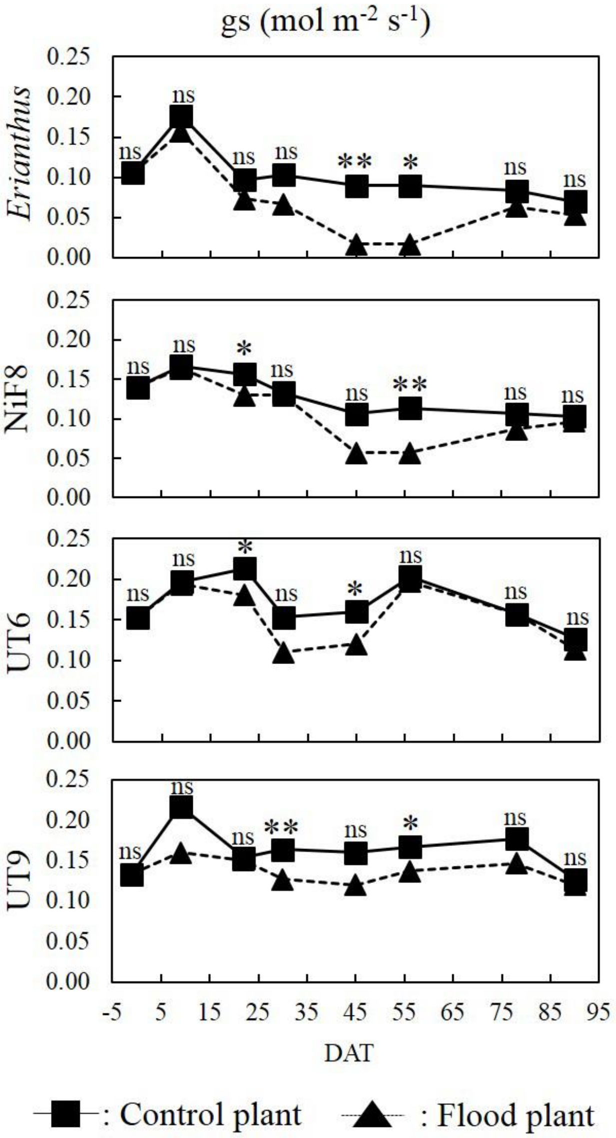

Fig. 3.3 Changing in stomatal conductance (gs) of control and flood plants during flood (1–60 days) and after drained (61–90 days) of 3 sugarcane cultivars as NiF8, UT6, and UT9 and *Erianthus* at 1 d before treatment, 9, 22, 30, 45, 56, 78 and 90 DAT.

Note: *, ** indicate significant effect in each period at $P < 0.05$ and 0.01, respectively and ns indicate not significant (n=3). DAT: days after treatment.

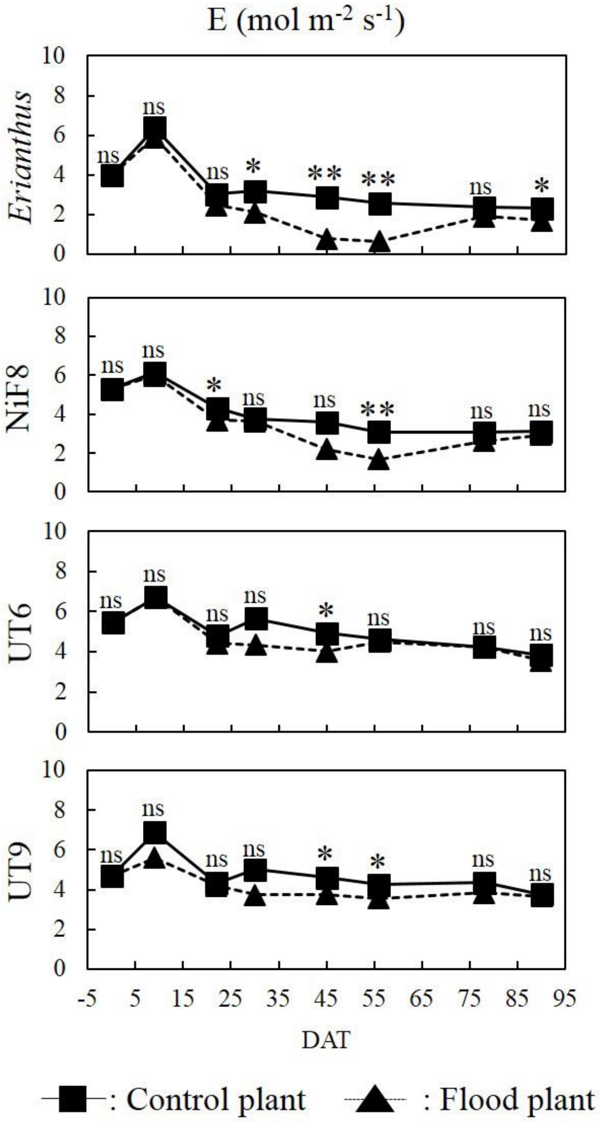

Fig. 3.4 Changing in transpiration (E) of control and flood plants during flood (1–60 days) and after drained (61–90 days) of 3 sugarcane cultivars as NiF8, UT6, and UT9 and *Erianthus* at 1 d before treatment, 9, 22, 30, 45, 56, 78 and 90 DAT.

Note: *, ** indicate significant effect in each period at $P < 0.05$ and 0.01, respectively and ns indicate not significant (n=3). DAT: days after treatment.

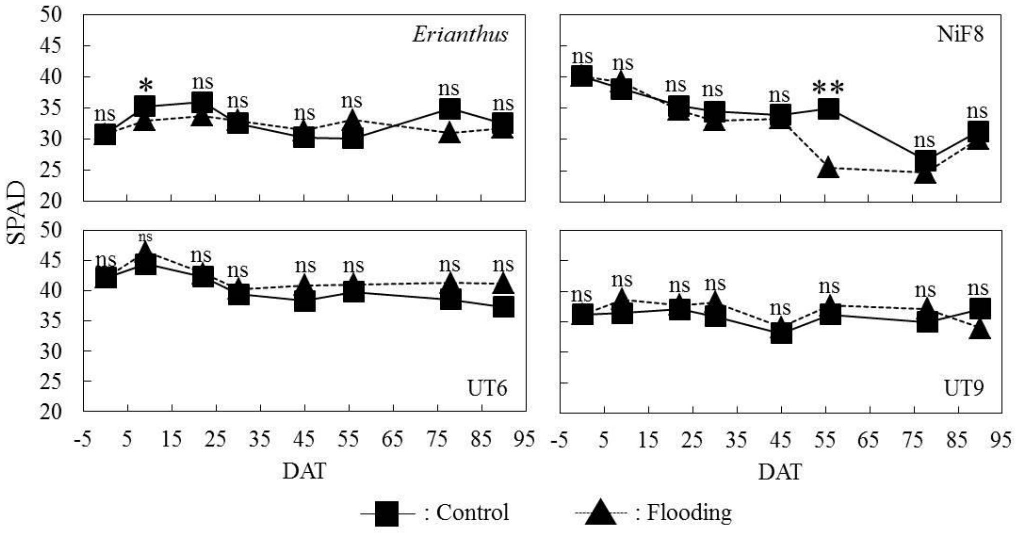

Fig. 3.5 Changes in SPAD value during flooding (1–60 days) and after draining (61–90 days).

Note: * and ** indicate significant differences between the control and flooded plants at $P < 0.05$ and 0.01, respectively and ns not significant (n=3).

Table 3.1 Effects of flooding on LA, leaf dry weight, stem weight, stem length, number of node, internode length, stem diameter, root dry weight, adventitious root dry weight and total dry weight at 60 DAT.

Cultivar	LA		Leaf dry weight		Stem weight		Stem length		Number of node	
	Control	Flood	Control	Flood	Control	Flood	Control	Flood	Control	Flood
	cm^{2} stalk^{-1}		g stalk^{-1}		g stalk^{-1}		cm stalk^{-1}		node stalk^{-1}	
Erianthus	-	-	-	-	81.1a	69.3a	145.3a	132.6a	13.6a	13.0a
NiF8	4,277a	3,812a	57.6a	48.4a	946.0a	897.0a	189.0a	184.6a	19.3a	19.0a
UT6	3,664a	3,447a	48.6a	43.1b	732.6a	735.0a	131.0a	131.0a	15.6a	13.3a
UT9	3,940a	3,289a	47.7a	40.2a	673.7a	604.0a	109.6a	98.6a	15.0a	13.6a

Cultivar	Internode length		Diameter		Root dry weight		Submerge nodes	Adventitious root dry weight		Total dry weight	
	Control	Flood	Control	Flood	Control	Flood		Control	Flood	Control	Flood
	cm stalk^{-1}		mm		g		node stalk^{-1}	g stalk^{-1}		g	
Erianthus	10.6a	10.2a	10.6a	9.7a	184.7a	132.0b	3.0	0	0.4	653.3a	628.4a
NiF8	9.8a	9.7a	24.6a	23.3b	47.9a	42.3a	3.0	0	1.9	337.1a	320.8a
UT6	8.3a	9.9a	28.0a	27.7a	46.6a	26.1b	6.0	0	6.3	259.9a	249.4a
UT9	7.3a	7.2a	28.3a	28.4a	32.5a	27.5a	5.3	0	1.9	205.9a	209.9a

Note: Means followed by the same lower case are not statistically different at $P < 0.05$ between the control and flooding treatments (n=3). The units of root dry weight and total dry weight of the sugarcane cultivars are expressed as g stalk^{-1} and those of *Erianthus* as g plant^{-1}.

Table 3.2 Effects of flooding on LA, leaf dry weight, stem weight, stem length, number of node, internode length, stem diameter, root dry weight, adventitious root dry weight and total dry weight at 90 DAT.

Cultivar	LA		Leaf dry weight		Stem weight		Stem length		Number of node	
	Control	Flood	Control	Flood	Control	Flood	Control	Flood	Control	Flood
	cm^{2} stalk^{-1}		g stalk^{-1}		g stalk^{-1}		cm stalk^{-1}		node stalk^{-1}	
Erianthus	288a	231a	3.0a	2.8a	85.6a	87.3a	148.0a	155.3a	13.0a	13.0a
NiF8	4,587a	4,143a	61.5a	54.6a	1,028.7a	971.6a	203.3a	190.6a	20.6a	17.6a
UT6	4,315a	3,582b	56.8a	46.1b	874.6a	804.0a	156.0a	149.3a	17.0a	15.6a
UT9	4,420a	3,987a	52.4a	47.1a	746.3a	723.0a	130.3a	118.3b	18.3a	15.0b

Cultivar	Internode length		Diameter		Root dry weight		Submerge nodes	Adventitious root dry weight		Total dry weight	
	Control	Flood	Control	Flood	Control	Flood		Control	Flood	Control	Flood
	cm stalk^{-1}		mm		g stalk^{-1}		node stalk^{-1}	g stalk^{-1}		g stalk^{-1}	
Erianthus	11.6a	11.9a	9.9a	10.7a	133.0a	90. 0b	-	0	0.5	670.7a	652.2a
NiF8	9.9a	10.7a	25.2a	25.1a	44.2a	34.8b	-	0	1.7	373.5a	387.8a
UT6	9.2a	9.5a	27.8a	26.8a	57.9a	29.1b	-	0	3.9	331.1a	309.6a
UT9	7.1a	7.7a	27.6a	28.2a	47.7a	35.3a	-	0	2.3	254.5a	276.9a

Note: Means followed by the same lower case are not statistically different at $P <$ 0.05 between the control and flooding treatments (n=3). The units of root dry weight and total dry weight of the sugarcane cultivars are expressed as g stalk^{-1} and those of *Erianthus* as g plant^{-1}.

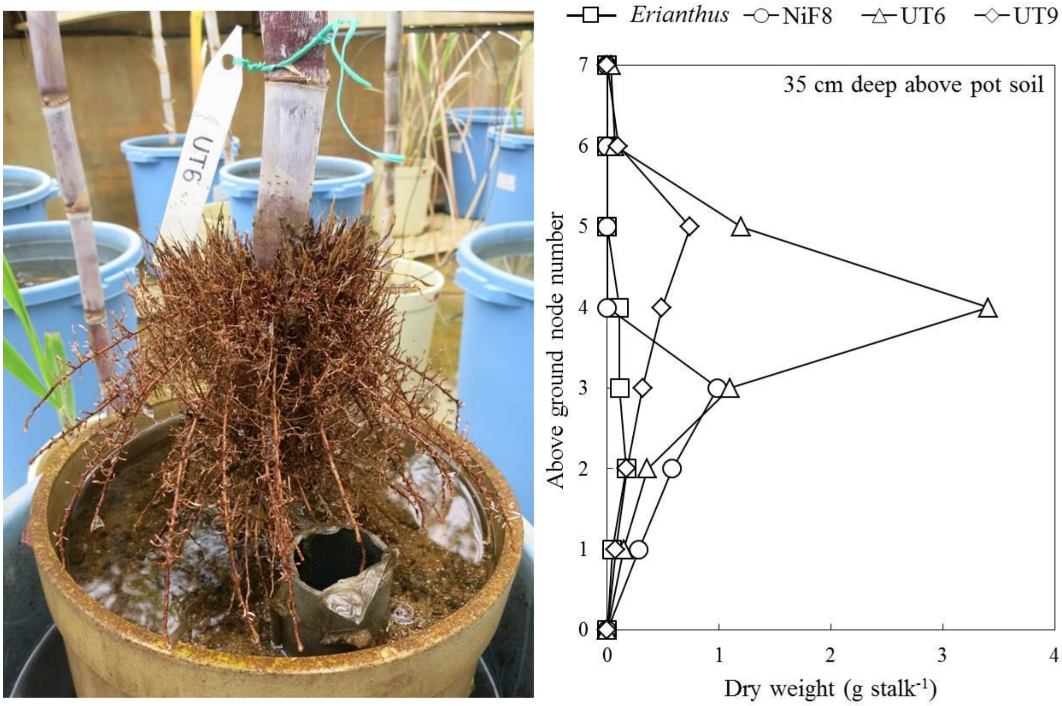

Fig. 3.6 Adventitious root development of UT6 (left) and dry weight of adventitious root at different nodes above the ground under water (35 cm deep above pot soil) (right).

Fig. 3.7 Growth and development of adventitious root under flooding.

Table 3.3 Effects of flooding on sucrose content in press juice and sugar yield.

Cultivars 60 DAT	Sucrose		Sugar yield	
	Control	Flood	Control	Flood
	(%)		g stalk^{-1}	
Erianthus	4.3b	7.3a	1.7a	2.6a
NiF8	22.9a	23.1a	110.7a	103.8a
UT6	19.6a	18.6b	71.7a	68.4a
UT9	17.9a	18.2a	60.5a	55.1a

Cultivars 90 DAT	Sucrose		Sugar yield	
	Control	Flood	Control	Flood
	(%)		g stalk^{-1}	
Erianthus	6.6a	6.2a	2.9a	2.7a
NiF8	24.7a	24.5a	127.1a	119.1a
UT6	21.3a	20.7a	93.1a	83.0a
UT9	19.1a	19.0a	70.9a	68.9a

Note: Means followed by the same lower case are not statistically different at $P < 0.05$ between the control and flooding treatments (n=3).

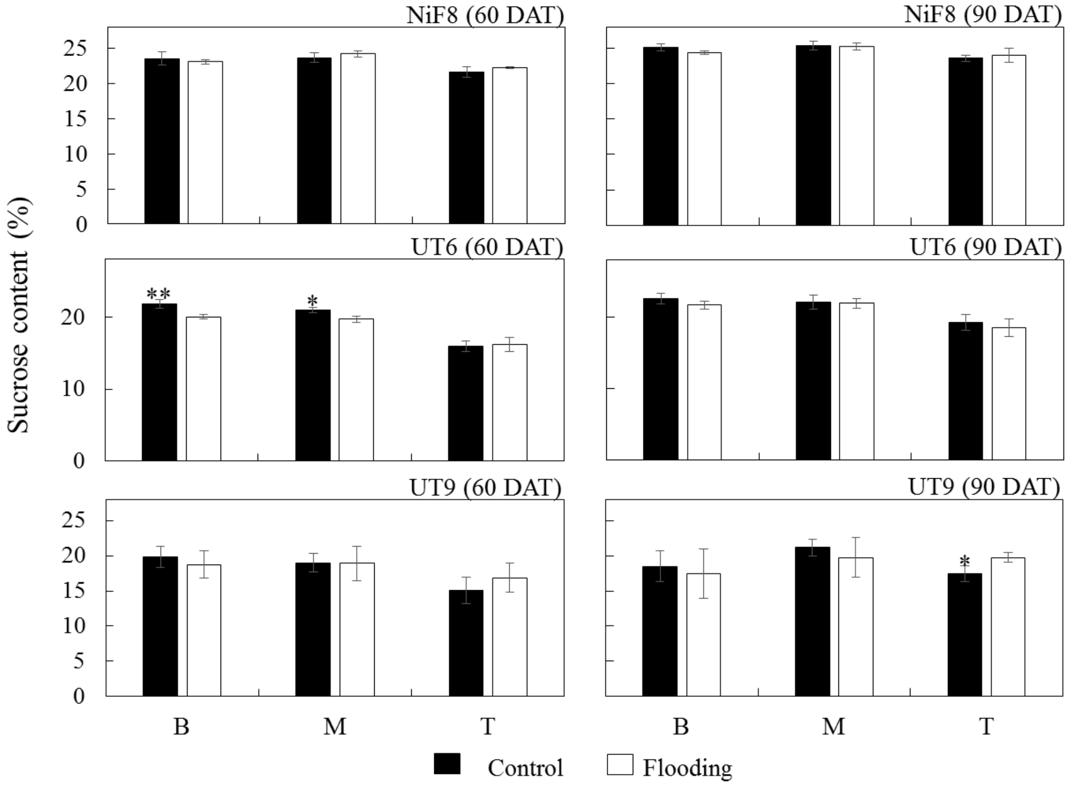

Fig. 3.8 Sucrose content in sugarcane juice of the three different stem parts.

Note: Bars show SD. * and ** indicate significant differences between the control and flooded plants at P < 0.05 and 0.01, respectively and ns not significant (n=3)

3.5 Discussion

In the present study, the decrease in *A* in flooded plants concurred with the results of a previous study that explained the decrease in *A* as being because of the slow diffusion of CO_2 in water and the decreased availability of light, resulting in a decreased flow rate of assimilates to the root. However, the decrease in *A* was dependent on many factors such as genotypes, environmental conditions, stage of growth, and duration of stress (Gomathi et al., 2014). All flooded sugarcane cultivars and *Erianthus* showed a gradual decrease in *A* during flooding (Fig. 2). The results of the present study were consistent with the findings of previous studies that 30 days flooding with 35 cm deep above the soil did not affect *A* of NiF8 cultivar (Jaiphong et al., 2016), while the 7 days periodic cycle (during Feb–Aug) showed an unaffected *A to* Ho 01-12 (energy cane), HoCP 96-540 (sugarcane), and L99-226 (sugarcane) but decreased *A* of L79-1002 (energy cane) by 50 and 48% in plant cane and ratoon cane, respectively (Viator et al., 2012). Furthermore, these results were in accordance with a previous study that reported a neutral or positive response of *A* to periodic 7 d flooding (Glaz et al., 2004a) while the periodic flooding cycle caused a neutral or positive response, as plants continued transpiration (Chabot et al., 2002). SPAD value was gradually decreased when flooding was prolonged in NiF8, however, the reduction of *A* by flooding was not only associated with the decreasing SPAD value considering that that of *Erianthus* was not decreased in the present study, whereas *A* was greatly decreased under a flooding condition.

After flooding, one of the immediate effects was the absence of oxygen, and a change from aerobic to anaerobic environment affected the growth and functions of roots. On 60 days after flooding, some roots showed symptoms of blackening and rotting. The data revealed that flooding decreased the root dry weight in all sugarcane cultivars and *Erianthus*, particularly in UT6 where root dry weight was decreased down to 44%. These results were consistent with a previous study that, in the absence of oxygen, root hairs died and eventually turned to blacken and rot, resulting in the entire underground root being choked, and root respiration also being impaired, thereby affecting important metabolic activities of plants (Gomathi et al., 2014). Sugarcanes and *Erianthus* compensated for the original root death by producing adventitious roots that emerged from the root primordia at nodes under the water and from aerial nodes by increasing numbers during prolonged flooding. It has previously been reported that flooding decreased the primary root weight, whereas plants stimulated adventitious roots with well-developed aerenchyma (Gilbert et al., 2008). These roots developed as a result of the hormonal imbalance that is induced by hypoxia in the submerged tissue, and were located in the upper layer of water, which has higher oxygen content (Gomathi et al., 2014). Three types of roots were produced after flooding: 1) those that emerged from the nodes that were located under the water; 2) those that developed from these first roots and grew upward against gravity, and; 3) those that emerged from aerial nodes above the water (Hidaka & Karim, 2007; Jaiphong et al., 2016). These data can explain that UT6 developed the number of roots and root size and length particularly in secondary roots. NiF8 and UT9 had similar characteristics of root growth, whereas *Erianthus* had a low dry weight and the number of roots at the node above the ground under water, which developed slowly (Figs. 3.6, 3.7). Subsequently, a high percentage of original root death of UT6 may have resulted from development of adventitious roots to maintain plant activity and survive under a flooding condition, while the other sugarcane cultivars and *Erianthus* showed lower percentage of original root death and lower quantity of adventitious roots.

After flooding for 60 days, the symptoms by flooding were not clearly observed. Leaves remained green, except NiF8 in which the lower leaves turned yellow during prolong flooding. However, LA decreased in all the sugarcane cultivars and *Erianthus*. These decreases may have resulted from the compensation by adventitious roots adopting the function of the original roots. A previous study reported that plant growth was inhibited due to the lack of nutrition and water uptake, whereas the development of adventitious roots and aerenchyma may have helped plants maintain water and nutrient uptake; moreover, these roots adapted better to flooding than the original roots (Begum et al., 2013; Gomathi et al., 2014; Laan et al., 1991). Plants produced roots, and ethylene-dependent death and lysis formed continuous gas-filled channels (aerenchyma) to assist plants maintaining root activity and supplying the necessary oxygen (Drew, 1997). Because of the oxygen concentration of the root zone, internal aeration in plants may be achieved by increasing the root porosity (Gomathi et al., 2014). Plants exposed to 60 days of flooding showed a slightly decreased growth rate in all the sugarcane cultivars and *Erianthus*. This result was related to the findings of previous studies that flooding inhibited leaf expansion and decreased LA, LAI, and leaf weight (Gilbert et al., 2007; Gomathi et al., 2014; Jaiphong et al., 2016), whereas flooding for < 3 month less damaged LAI (Gilbert et al., 2008). Flooding also decreased stem weight on 90 DAT, which is consistent with a previous finding that waterlogging over 15–60 days at the grand growth phase decreased yield by approximately 5–30% because of the lack of nutrition and water uptake (Gomathi et al., 2014), while 3 months of flooding decreased the yield by 18–37% in plant cane and 61–63% in a second ratoon (Gilbert et al., 2008). However, growth and yield loss may depend on the tolerance of the cultivar, as it has been shown that there is loss in yield in CP 95-1376 but not in CP 95-1429 (Glaz et al., 2004b), whereas high water table had no effect on yields of CP 72-2086 and CP 82-1172 but adversely affected CP 80-1743, resulting in a decrease in yield by 25.1% (Glaz et al., 2002). Thus, plants may require more time to recover their root system and growth.

From this study, each of the sugarcane cultivars was thought to have their advantages to survive under a flood condition. Sugar yield, which is the most important factor for sugar refinery, was lowered by flooding in all the cultivars on 90 DAT; however, the decreases were not statistically significant, suggesting flooding for 60 days at this growth stage had little effects on sugarcane. These results are supported by previous studies that 30 days flooding did not affect sucrose content in the flooded plants of NiF8 cultivar compared to the control plants (Jaiphong et al., 2016); 2 days of periodic flooding in each of eight 14 days cycles increased cane and sucrose yield in the CP 72-2086 and CP 80-1827 cultivars (Glaz & Gilbert, 2006); and sugar yields were not affected in CP 72-1210 when grown in a high water table (Pitts et al., 1990). We could not confirm clear effects of *Erianthus* on flooding tolerance in this study, but it would be worth using *Erianthus* as a genetic resource since it had higher sucrose content and sugar yield than control on 60 DAT.

3.6 Conclusion

Our study demonstrated that flooding affected CO_2 assimilation in all sugarcane cultivars and *Erianthus* to different extents. *Erianthus* decreased CO_2 assimilation during flooding, which was greatly decreased when flooding was prolonged, whereas that of NiF8 was decreased when flooding was prolonged, and that of UT6 and UT9 were slightly decreased to lower than the control during flooding. After draining, CO_2 assimilation was increased in all cultivars, but remained lower than that of the control. During flooding, the original root damaged results in an arrest of root activities and effected growth both during and post-flood. Consequently, plants compensated for damaged roots during flooding by the development of adventitious roots in all cultivars. During the post-flood, adventitious roots were not functional, whereas the original root remained damage and continued to affect the growth of plants. Therefore, plants may require more time to recover their roots and growth after flooding. The result indicated that 60 days of flooding has fewer effects on the growth of all sugarcane cultivars and *Erianthus*. However, after draining, damaged roots may result in growth remaining lower than that of the control. Sucrose content was not affect by flooding except in UT6. However, sucrose content recovered after draining. Sodium content in juice was increased in flooded plants of all cultivars, mainly at the bottom of stem, and remained high, even after water was drained and re-irrigation occurred. Consequently, the varying extent of formation of adventitious roots within each cultivar may result in differences in the compensation for original root death. The high quantity and well-developed aerenchyma of the root may assist plants to survive and maintain growth and yield during flooding. However, growth after flooding is most important for yield and quality. Consequently, the tolerance of a cultivar could be associated with post-flood compensatory ability. The duration of recovery after flooding should be studied to obtain an optimal yield and quality at harvesting.

Chapter 4

General discussion

Sugarcane play a major role in economic crop in Thailand. Sugar industry was increased dramatically consistent with an increase of sugarcane production. Increased of sugarcane price and encourage from government caused sugarcane plantation, total sugarcane production and sugar production increased 54.70, 24.80 and 63.76%, respectively within 5 year from 2009/10–2014/15 (Pipat, 2015; USDA Foreign Agriculture Service, 2010). For this reason, Thailand become one of the world's top five producers, and become the second largest sugar exporter on the world sugar market (SUGAR Expertise, 2015). However, even sugarcane plantation, total sugarcane production and sugar production increased but on the other hand production per area was reduced 10.48% per hectare (Pipat, 2015). Decreased of production per area may from many factor as insufficient of irrigation that available only 10% of total sugarcane plantation, soil fertility and variety that inappropriate to each area. In additional, due to the global climate change leading to uncertain of weather in Thailand that cause changes in rain fall pattern result in drought or flood were possible in every year with difficult to predictable.

Furthermore, according to the government encouraging rice producers to switch to sugarcane production for better returns result in increased of sugarcane plantation, meanwhile it may increase the risk of floods and cause problems for cane growers, particularly in the central basin in rainy season.

Sugarcane production in Thailand was frequent to face with drought and flood even in the rainy season that start from May to September, there are two rainfall peaks in May and August which sometime problem with serve flood, while there was a dry spell in July result in drought stress. During rainy season, plants that planting in the early of rainy season (March–May) have 4-6 months age or grow in the grand growth phase or during 4-10 months which is important for actual cane formation, elongation, and accumulation of yield (Gascho, 1985). Then, drought and flood stress during grand growth phase in rainy season may effect to growth and result to low yield at harvesting.

The present study have 2 experiment as 1) effects of duration and combination of drought and flood conditions on leaf photosynthesis, growth and sugar accumulation in sugarcane and 2) Changes in Photosynthesis, Growth, and Sugar accumulation of Commercial Sugarcane Cultivars and *Erianthus* under Flood Conditions. This study involved series of experiment that focused on effect of duration and combination of drought and flood and including with study in different varietal of sugarcane and *Erianthus* during grand growth phase or 6 months age. The experiment was using a pot experiment and conduct in a glasshouse since germinated until finish experiment.

4.1 Leaf photosynthesis characteristic and their response to environmental stress

4.1.1 Effects of duration and combination of drought and flood conditions to leaf photosynthesis

The characteristic of photosynthesis in this study demonstrated plants reduced *A* in both drought and flood condition. Plants under drought condition, *A* and E ceased in accordance with the stomatal closure. Drought reduced *A* dramatically lower than control and flood plants and continued lower when drought was prolong. This finding relate to previous study that reported, due to the stomata closing to prevent transpiration loss, which reduced the amount of CO_2 required for photosynthesis (Koonjah et al., 2006; Cornic and Massacci, 1996). However, drought plants increased *A*, E and gs again after re-irrigation but maintained slightly lower than control (Fig. 2.9).

Under flood and prolong flood (15 and 30 days) reduced *A* with slightly lower than control during flood and maintained lower even water was drained and re-irrigated. However, this study found the positive effect with an increased E and gs in the early stage of flood (7 days), while *A* was not different with control. Less effect of flood in this study may from the adventitious root that emerged after flood and related to previous study that explained the development of adventitious roots and aerenchyma may assist plants to continue with water and nutrient uptake; moreover, these roots were better adapted to flooding than the original roots (Begum et al., 2013; Gomathi et al., 2014; Laan et al., 1991) (Fig. 2.9).

Combination treatments demonstrated photosynthesis was unaffected when the soil was dehydrated after flood (F + PD and PF + PD). The *A* maintained high during flood cause of from the adventitious root compensated root damaged. While after changed to prolonged drought condition *A* also maintain high that may result from plants used the water from soil that still have high moisture content after drained together with water from irrigated for drought treatment (15% (v/v) soil moisture) per day. Then, the effect of drought after flood may less than effect in the treatment that started from drought due to high moisture content still in the soil (Fig. 2.9).

However, plants in drought, flood and combination treatment maintained decrease in photosynthesis lower than control even changed to normal condition because of the destroyed of underground roots were not recovered and leading to a deficiency in the absorption of water and nutrients. While, adventitious root in flood treatments were dried and not functional. Thus, plants may require more time and proper soil conditions to recover their root system and growth (Fig. 2.6).

Drought conditions reduced SPAD, and it was found that these effects may continue even after re-irrigation. By contrast, SPAD was maintained at a fairly high level for all other treatments (flood, prolonged flood, F + PD, and PF + PD) result from adventitious roots and aerenchyma may assist plants to continue with water and nutrient uptake during flood.

4.1.2 Changes in photosynthesis of commercial sugarcane cultivars and *Erianthus* under flood conditions

This study focused on prolonged flood (60 days) and compared with 3 commercial sugarcane cultivars as NiF8, UT6 and UT9 and *Erianthus* spp. The different cultivars showed the different characteristic of photosynthesis during flood and post flood. All flooded sugarcane cultivars and *Erianthus* showed a gradual decrease in *A* during flooding. However, it seem 60 days flooded may less effect to *A*, gs and E of UT6 and UT9 while NiF8 affected when prolonged flood. These result of tolerance may from 3 of sugarcane cultivars produced a lot of adventitious root that help plant to compensated with underground root death, whereas *Erianthus* produced a small number of roots and not develop as other sugarcane cultivars, result in dramatically decreased of *A* since flood.

After re-irrigated, all sugarcane cultivars and *Erianthus* have the same result of first experiment by increased *A*, gs and E but maintained lower than control. These result may consist with the first experiment that original root were destroyed and not recovered even 1 month past after drained and re-irrigated (Table3.2).

SPAD was gradually decreased when flooding was prolonged in NiF8, whereas SPAD values of UT6 and UT9 were not different from the control during flooding. By contrast, SPAD of *Erianthus* was not decreased in the present study, whereas *A* was decreased under a flooded condition.

4.2 Effect of drought and flood to root and their response under environment stress

4.2.1 Effects of duration and combination of drought and flood conditions to root growth and development

Drought and prolonged drought condition reduced root growth under stress in term of root weight and quantity of root, however, root weight remained lower than control even plants were re-irrigated. While, roots of plants that were exposed to flood and prolonged flood conditions demonstrated the root hairs died and the original roots became blackened and rotten caused of reduced in root weight more than control and drought condition. Furthermore, even drained and re-irrigated underground root remained damaged, especially in prolong flood.

Reduction of root under drought and flood condition result in reduced of photosynthesis due to improper root system absorption of water is hampered result in “physiological drought” (Gomathi et al., 2014) and the plant leave assume a tightly curved position similar to drought stress. Moreover, insufficient of root will effect to water and nutrient absorption leading to nutrient deficiency and effect to plant growth. After drought and flood, slow recovery of root may result in reduce growth, yield and quality of sugarcane.

4.2.2 Effect of prolog flood condition to root in commercial sugarcane cultivars and *Erianthus*

Prolonged flood effect to every sugarcane cultivars and *Erianthus*, but among the cultivars, root of UT6 was highest damaged (Table 3.1). Meanwhile, UT6 produced highest of adventitious root among the cultivars and help plant to compensated original root death during flood by maintained high photosynthesis rate and growth under flood condition. However, high damaged of root may cause of sucrose reduced in UT6 while the other cultivar were not. By contrast, *Erianthus* that also had high damage of root but produced a few number of adventitious root had reduced of photosynthesis dramatically after flood and reduced total dry weight (Table 3.1). While, UT9 and NiF8 had root destroyed lower than 20% and produced similar adventitious root weight had less effect from prolong flood to photosynthesis, growth and sucrose content. Therefore, adventitious roots is essential for plant under flood condition to maintain their growth as UT6 while *Erianthus* was effect.

However, after flood, the cultivar that rapid recover their root may advantage for plant to recovery their growth. In this study, all sugarcane cultivars and *Erianthus* able to survive under flood condition by adventitious but after drained adventitious root were not functional and dried. While, original root remained damaged resulting in flooded plant trend to reduced their photosynthesis, growth and sucrose accumulation. Then, the cultivar that could be use in flood area should be associated with post-flood compensatory ability, not only adventitious root but important is root recovery.

4.3 Effect of drought and flood to sugarcane growth

4.3.1 Effects of duration and combination of drought and flood conditions to sugarcane growth

Drought and prolonged drought plants exhibited a yellowing of the lower leaves, which proceeded to the upper leaves when drought conditions were extended. Plants reduced LA, stem and total dry weight due to reduction in light interception, plant extension rate, and photosynthesis. Furthermore, even re-irrigated plants had a lower LA than control plants and effect to stem and total dry weight. In this study, plant may use more than 1 month with irrigation to recovery their growth as stem weight and total dry mass, while LA remained lower. By contrast, flooded plants, including flood, prolonged flood, and combination (F + PD and PF + PD) treatments, did not exhibit any change in leaf color, but LA did slightly decrease, stem and total dry weight increased in a similar manner to control plants. This result may related to adventitious root that emerged during flood to continue to take up water and nutrients, and may have offset any losses associated with flooding.

4.3.2 Effect of prolog flood condition to growth in commercial sugarcane cultivars and *Erianthus*

The LA slightly reduced in all sugarcane cultivars when flood, and even after flood and re-irrigation for 30 days, LA remained lower but effect in UT6 while, plant growth were not affect but slightly lower than control. Limit of LA result in lower photosynthesis in flood plant compared to control and related to reduction of plant growth. Thus, the decrease or limitation of shoot growth after draining may have been caused because of damaged original root that remained unrecovered, whereas adventitious roots were not functional. Therefore, to recover their root function after flooding, plants may require a longer time than that required to recover their growth.

4.4 Effects of drought and flood to sucrose accumulation

4.4.1 Effects of duration and combination of drought and flood conditions to sucrose accumulation

Short drought may advantage to increased sucrose content but not for prolong drought relate to previous study that reported that sucrose content increased during a period of low rainfall or under dryland conditions but was reduced under extreme drought conditions (Robertson et.al., 1999a); and similarly, a 6-wk drought during the grand growth phase was found to reduce the sucrose content by an average of 4.7% and sugar yield by 11.7%–19.1% (Wiedenfeld, 2000). However, after re-irrigated, sucrose content increased but it may take approximately 1 month in normal condition to recovery and similar to control. Flood and prolonged flood decreased sucrose content in in the early stage of flood then dramatically increased even in prolonged flood to reach levels that were similar to the control plants. While, combination of flood and drought demonstrated F+PD induced high sucrose accumulation after flood, whereas PF+PD was slow increased after prolonged flood but not different with other treatment after re-irrigated. Then, flood less than 1 month may not effect to sucrose content even follow by drought or prolong drought that also less than 1 month. However, the result of long duration of flood were explain in the second experiment.

4.4.2 Effect of prolog flood condition to sucrose accumulation in commercial sugarcane cultivars and *Erianthus*

Prolong flood increased sucrose content in *Erianthus*, NiF8 and UT9 during flood slightly higher than control, result was similar 30 days flood in first experiment. By contrast, only UT6 that reduced sucrose content. However, at 30 days after drained and re irrigation their have no different in all cultivar, but flood plants of all cultivars trend to be reduce sucrose content by slightly lower than control. This reduction of sucrose content similar trend of growth reduction after prolonged flood in this experiment. Then, early harvesting after prolong flood may obtain low yield and sucrose content. Therefore, to recover their root function after prolonged flood, plants may require a longer time than that required to recover their growth and sucrose content.

4.5 Analysis of the concentration of various ions in sugarcane juice

Both of experiment found the concentration of sodium (Na^+) was higher in the flooded plants than in the control and drought plants. Furthermore, sodium content remained high in flooded plants, even after water was drained and conditions returned to normal condition. The highest sodium content was found in the bottom part of the stem, whereas the middle and top parts of the stem had similar sodium concentrations to the control plants. However, there is still a lack of information about this increase in sodium content in the juice of flooded sugarcane plants, and so its role in flood tolerance remains unclear. Thus, further study on the relationship between sodium accumulation and physiological changes under flood conditions may help to explain this phenomenon in the future.

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Summary

This study was studies on effects of extreme biotic stress as drought and flood to sugarcane during grand growth phase. Pot experiment was conducted in glasshouse and set the planting date similar to planting schedule in Thailand (March–April). The experiment were set when sugarcane was 6 months after planted that match to late of rainy season (Mid of May–Mid of October) connect to early of dry season (Mid of October–February) in Thailand. In actually, during this time sugarcane in Thailand is risk to face with severe flood and drought or combination stress in every year, before harvesting season that will be start from mid of November to end of Mach which related to sugar mill. Thus, the information of yield and quality after stress is still lacking before determine to harvest. This study aimed to investigate the effect of biotic stress to growth, yield and quality during and after stress for more information for the advantage to crop management in the future.

The objective of this study is to determine the effects of duration and combination of drought and flood to photosynthesis, growth, yield and quality. Furthermore, study on prolong flood condition effects to photosynthesis, growth, and sugar content of commercial sugarcane cultivars and *Erianthus* were examine for more understanding of their growth, adaptability and quality after stress.

Effects of duration and combination of drought and flood conditions on leaf photosynthesis, growth and sugar accumulation in sugarcane

The objective of this study was to investigate the effects of various combinations of drought and flood of varying duration on sugarcane growth and yield during the grand growth phase of sugarcane cultivar NiF8. In addition, morphological and chemical changes in the sugarcane juice and adventitious roots were analyzed to gain a better understanding of their adaptive significance.

This study found, *A* was reduced dramatically in sugarcane plants that were exposed to drought and prolonged drought treatments due to the stomata closing to prevent transpiration loss, which reduced the amount of CO_2 required for photosynthesis. However, *A* was increased once the plants were re-irrigated but remained slightly lower than control plants. By contrast, the flood and prolonged flood and included combination treatments only had slightly affected the photosynthesis of the plants by slightly reduced *A*, even when the water was drained or the plants were subsequently exposed to prolonged drought in combination treatment.

Plants that were exposed to flood, adventitious roots with well-developed aerenchyma were produced, the number of which increased under prolonged flooding, which may help plants to compensated original root death that losses associated with flooding. The expansion of LA was interrupted by drought since drought was started and remained at a low level compared to control and flood even after re-irrigation. Flood and combination treatment were slightly reduced LA during flood, then increased once the flood and combination treatments had finished. Drought and prolonged drought result in reduction of stem and total dry weight remained lower compared to control and flood even after re-irrigated. On the other hand, the flood and combination treatments were increased similar to control. Drought reduced root growth by reduced of root weight result in insufficient and inadequate root system absorption of nutrient and water is serious effect plant growth when prolonged drought. However roots were recovered once the plants were re-irrigated. While, root of plants that were exposed to flood and combination treatments were damaged more than by drought, root hairs died and the original roots became blackened and rotten, leading to the arrest of root respiration and affecting important metabolic activities of the plants. Nevertheless, plants were recovered after drained and re-irrigate.

The sucrose content of the sugarcane juice increased during the early stages of drought but then decreased under prolonged drought conditions when the plants were placed under extreme stress. However, sucrose content increased again following re-irrigation. In plants that were exposed to the flood and combination treatments, the sucrose content decreased in the early stages of flooding and then dramatically increased to reach levels that were similar to the control plants, even during prolonged flooding or where flooding was followed by drought.

At the time of harvest, there were no significant differences in stem fresh weight, sucrose content, or sugar yield between treatments. Therefore, it is concluded that drought and prolong drought affected to plant photosynthesis, growth and sucrose content more than plants that exposed to flood. Flood plant that produced adventitious roots with well develop of aerenchyma may compensate the negative effects of root death and continue to take up water and nutrients and help plants to maintain their growth, yield and sucrose content during a flood. However, in this study flood for 15–

30 days may less effect to sugarcane growth, then study on prolong flood and post flood is necessary to obtain more information of flood effects.

Changes in photosynthesis, growth, and sugar accumulation of commercial sugarcane cultivars and *Erianthus* under flood conditions

The objective of the present study was to determine changes in photosynthesis, growth, and sugar content in three commercial sugarcane cultivars and *Erianthus* under long-term flood conditions and after the change to normal conditions. Moreover, morphological changes and development of adventitious roots were analyzed for gaining a better understanding of its adaptive significance of tolerance.

This study demonstrated that flooding affected *A* in all sugarcane cultivars and *Erianthus* to different extents. *Erianthus* decreased *A* during flooding, which was greatly decreased when flooding was prolonged, whereas that of NiF8 was decreased when flooding was prolonged, and that of UT6 and UT9 were slightly decreased to lower than the control during flooding. After draining, *A* assimilation was increased in all cultivars, but remained lower than that of the control.

At 60 days after flooded, some roots were observed to blacken and rot. Flooding decreased the root dry weight in all sugarcane cultivars and *Erianthus*, particularly in UT6 where root dry weight highest decreased. Sugarcane and *Erianthus* compensated for the original root death by producing adventitious roots that emerged from the root primordia at nodes under the water and from aerial nodes, with increased numbers when prolonged flood had occurred. This present study demonstrated that UT6 was most developed in root size and length, and particularly developed secondary roots.

NiF8 and UT9 had similar characteristic root growth, whereas *Erianthus* had a low number of roots which developed slowly. High percentage of original root death of UT6 may have been compensated for by increased development of adventitious roots to maintain plant activity and survival under a flooded condition. At 60 days of flooded, the symptoms of flooding were not clearly visible. Leaves remained green, except in NiF8 where the lower leaves turned yellow during prolong flooding, and LA, stem weight, and stem length were slightly decreased in all cultivars. These decreased effects may have resulted from the compensation by adventitious roots adopting the function of the original root.

At 30 days after drained, roots were observed and found underground roots remained injured from flooded, with root dry weight remaining lower than that of the control in all sugarcane cultivars and *Erianthus* and still high affect to UT6. However, although most of the growth in flooded plants was not significantly different from the control but it remained lower. The decrease in growth continued because of the compromised root system leading to a deficiency in the absorption of water and nutrients. Thus, plants may require more time and proper soil conditions to recover their root system and growth.

At 60 days after flooded, flood did not affect the total sucrose content in *Erianthus*, NiF8, and UT9; however, the sucrose content in UT6 was affected. Furthermore, UT6 had lower sucrose content in the bottom and middle part of stem than in the control plant, whereas the other sugarcane cultivars and *Erianthus* were not different from the control. Flooding also affected the total sugar content in UT6, but did not affect the sugar yield related to stem weight. At 30 days after drained, there was no difference between the control and flooded plants in each cultivar for sucrose content, total sugar, and sugar yield.

This study demonstrated 60 days flood was less affect to all sugarcane cultivars and *Erianthus*. However, during flood, photosynthesis and plant growth were slightly reduced compare to control plants, while sucrose content was not affected except in UT6, due to the adventitious root help plants to compensated original root death that losses associated with flooding. This present study found the underground roots remained injured from flooded even after drained may problem and affect to plant growth and sucrose content trend to be decrease after flood. Consequently, the tolerance of a cultivar could be associated with post-flood compensatory ability as rapid root and shoot recovery from flood. Moreover, the duration of recovery after flooding should be studied to obtain an optimal yield and quality at harvesting.

Summary in Japanese

サトウキビ(*Saccharum* spp.)はタイの主要な作物のひとつであるが，近年は国策によりイネ作からより収益の多いサトウキビ生産への転換が奨励され，急激に栽培面積が増加した．その間，気候変動により地球温暖化が進み，深刻な洪水被害や干ばつが世界の多くの地域で頻発している．本研究の目的は，1)湛水，乾燥の期間およびその組み合わせがサトウキビの成長および収量に与える影響を調査すること，および 2) 長期の湛水条件が複数のサトウキビ品種およびエリアンサス属の成長および糖含量に与える影響を明らかにすることとした．この目的を達成するため，琉球大学農学部のガラスハウスでポット栽培試験を二度行った．実験 1（2013 年 4 月－2014 年 2 月）ではサトウキビ品種‘NiF8’を用い，定植後 6 カ月の植物体を乾燥および湛水処理した．処理区は，15 日間の乾燥処理区，30 日間の長期乾燥処理区，15 日間の湛水処理区，30 日間の長期湛水処理区，長期乾燥後に 15 日間湛水する区，長期乾燥後に長期湛水する区，対照区の計 7 処理区を設けた．対照区および乾燥区では土壌含水率がそれぞれ 50 および 20%（体積比）となるよう灌水し，湛水条件ではポリバケツにポットごと浸漬した．実験 2（2014 年 4 月－2015 年 2 月）では，サトウキビ品種（NiF8，UT6，UT9）およびエリアンサス属系統を用い，定植後 6 カ月の植物体を湛水処理し，長期的な湛水処理の影響を調査した．湛水処理は実験 1 と同様の方法で行った．湛水は処理後 60 日で排水し，その後 1 ケ月間対照区と同様に灌水を行った．両方の実験で，ガス交換特性，生育パラメーター，ショ糖含量および搾汁液の品質を調査した．

実験 1 の結果から，乾燥下では気孔閉鎖により光合成速度が減少したが湛水処理ではそのような影響は見られなかった．葉面積も同様に乾燥処理でのみ低下した．湛水後に乾燥処理を行うと，光合成速度および葉面積のいずれも減少した．湛水下では，地下部は損傷し，それを補償するため通気組織の良く発達した不定根が湛水浸漬された茎部より出現した．処理後 75 日後の茎新鮮重，ショ糖含量，糖収量に有意な処理区間差は見られなかった．実験 2 により，対照区と比較し，長期湛水処理区では，エリアンサスの光合成速度が低下し，サトウキビ品種の光合成速度はわずかに低下した．湛水 60 日後の成長パラメーターは対照区に比べ影響は小さく，UT6 以外の搾汁液のショ糖含量は変化しなかった．各品種により異なる傾向を見せたものの，実験 1 と同様に湛水による根の腐敗を補償し，生育を維持するように浸漬部より不定根が発生した．しかし，排水後 30 日でも根は損傷を受けたままで，不定根は乾燥・枯死するため，結果対照区と比べ生育が抑制された．一方で，ショ糖含量および糖収量に顕著な差は見られなかった．

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