

Diurnal Changes in Photosynthetic Parameters of Promising Sugarcane Clones under Drought Conditions and Rehydration at the Mature Growth Stage in Northeast Thailand

การเปลี่ยนแปลงในรอบวันของพารามิเตอร์การสังเคราะห์ด้วยแสงในอ้อยโคลนดีเด่น ภายใต้สภาวะขาดน้ำและการฟื้นตัวที่ระยะแก่ ในภาคตะวันออกเฉียงเหนือของประเทศไทย

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Abstract

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Understanding how sugarcane photosynthesis fluctuates throughout the day during drought and rehydration is vital for grasping how these plants adapt to challenging conditions. However, the knowledge on the diurnal change of photosynthesis of Thai sugarcane remains limited. Therefore, the aim of this study was to evaluate the diurnal changes in photosynthetic parameters under drought and after rehydration at mature growth stage of two promising sugarcane clones (KK07-599 and KK3/E09-1) and DOA KK3. The experiment was conducted at Khon Kaen Field Crops Research Center (KKFCRC), Khon Kaen Province, Thailand in 2023. Sugarcanes were planted in cement blocks with two irrigation managements: (1) well-watered based on plant requirement, and (2) restrict-watered for 19 days at 298–316 days after plantation (DAP) to induce water deficit, and re-watering at 317 DAPs to measured three days after rehydration at day 320. The results showed that the net photosynthesis rate, transpiration rate, and stomatal conductance of all sugarcane genotypes were reduced under drought conditions compared to well-watered condition. These parameters were lowest in the early morning and evening, reaching their highest levels in the morning or at noon. The net photosynthesis rate of well-watered group was $10\text{--}15 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, which was higher than that of the restrict-watered group (less than $10 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Conversely, the restrict-watered group showed the highest internal CO_2 concentration at dusk or dawn (approximately $300\text{--}400 \mu\text{mol mol}^{-1}$). The diurnal variation in photosynthetic parameters observed across all sugarcane genotypes did not reflect drought tolerance. However, following rehydration, the sugarcanes were able to restore its photosynthetic

parameters to levels comparable to those under well-watered conditions. This demonstrates the strong adaptability of sugarcane in rain-fed regions, where cultivation commonly occurs in the late rainy season and is followed by a prolonged drought before rainfall resumes. Nevertheless, further research into the source-sink relationship and sugar translocation is necessary to gain a deeper understanding of sugar accumulation under drought conditions.

Keywords: net photosynthesis rate, transpiration rate, stomatal conductance, drought stress, diurnal variation

บทคัดย่อ

ความเข้าใจเกี่ยวกับการเปลี่ยนแปลงในรอบวันของการสังเคราะห์ด้วยแสงในอ้อย ภายใต้สภาวะขาดน้ำและการฟื้นตัวหลังได้รับน้ำอีกครั้งมีความสำคัญต่อการรับทราบถึงกลไกการปรับตัวของพืชภายใต้สภาวะดังกล่าว อย่างไรก็ตาม ความรู้ด้านการสังเคราะห์ด้วยแสงในรอบวันของอ้อยในประเทศไทยยังมีจำกัด ดังนั้น การทดลองนี้จึงมีวัตถุประสงค์เพื่อประเมินการเปลี่ยนแปลงในรอบวันของพารามิเตอร์การสังเคราะห์ด้วยแสงของอ้อยโคลนดีเด่น (KK07-599 และ KK3/E09-1) และพันธุ์ กว.ก. ขอนแก่น 3 ที่ระยะแก่ ภายใต้สภาวะแล้งและการฟื้นตัวหลังได้รับน้ำอีกครั้ง ดำเนินการ ณ ศูนย์วิจัยพืชไร่ขอนแก่น จังหวัดขอนแก่น ปี พ.ศ. 2566 โดยปลูกอ้อยในวงบ่อซีเมนต์ด้วยการจัดการการให้น้ำ 2 รูปแบบ คือ (1) ให้น้ำตามความต้องการของพืช (2) งดการให้น้ำนาน 19 วัน (เมื่ออ้อยมีอายุ 298–316 วันหลังปลูก เพื่อชักนำการขาดน้ำ) และให้น้ำอีกครั้งเมื่ออ้อยมีอายุ 317 วันหลังปลูก เพื่อบันทึกข้อมูลการฟื้นตัวหลังการได้รับน้ำอีกครั้งในวันที่ 3 หรืออ้อยมีอายุ 320 วันหลังปลูก จากการศึกษาพบว่า การขาดน้ำทำให้อ้อยทุกพันธุ์/โคลนมีค่าอัตราการสังเคราะห์ด้วยแสงสุทธิ อัตราการคายน้ำ และค่าชักนำการเปิด-ปิดปากใบลดลงเมื่อเปรียบเทียบกับอ้อยที่ได้น้ำตามปกติ โดยค่าพารามิเตอร์ดังกล่าวมีค่าน้อยสุดในช่วงเช้าตรู่และช่วงเย็น แต่มีค่าสูงสุดในช่วงเช้าและช่วงกลางวัน ซึ่งค่าอัตราการสังเคราะห์ด้วยแสงสุทธิของอ้อยที่ได้รับน้ำตามปกติมีค่า $10\text{--}15\ \mu\text{mol CO}_2\ \text{m}^{-2}\ \text{s}^{-1}$ ขณะที่ กลุ่มอ้อยที่ขาดน้ำมีค่าน้อยกว่า $10\ \mu\text{mol CO}_2\ \text{m}^{-2}\ \text{s}^{-1}$ ทั้งนี้ พบว่า ค่าความเข้มข้นของคาร์บอนไดออกไซด์ในใบของอ้อยกลุ่มขาดน้ำมีค่ามากที่สุดในช่วงเช้าตรู่หรือช่วงพลบค่ำ (ประมาณ $300\text{--}400\ \mu\text{mol mol}^{-1}$) การเปลี่ยนแปลงในรอบวันของพารามิเตอร์การสังเคราะห์ด้วยแสงของอ้อยทุกพันธุ์/โคลนที่ดำเนินการศึกษาในครั้งนี้ไม่ได้บ่งชี้ถึงความต้านทานต่อสภาวะแล้ง แต่พบว่าเมื่ออ้อยได้รับน้ำอีกครั้งจะสามารถฟื้นฟูพารามิเตอร์การสังเคราะห์ด้วยแสงให้ใกล้เคียงกับกลุ่มอ้อยที่ได้รับน้ำตามปกติ ซึ่งแสดงให้เห็นถึงศักยภาพการปรับตัวของอ้อยในเขตอาศัยน้ำฝนที่ส่วนใหญ่นิยมเพาะปลูกในช่วงปลายฤดูฝนและต้องเผชิญกับสภาวะแล้งที่ยาวนานก่อนที่จะได้รับน้ำฝนตกอีกครั้ง นอกจากนี้ การศึกษาถึงความสัมพันธ์ระหว่าง source-sink และการลำเลียงสารอาหารจะช่วยสร้างความเข้าใจต่อการสะสมน้ำตาลภายใต้สภาวะแล้งของอ้อยได้มากยิ่งขึ้น

คำสำคัญ: อัตราการสังเคราะห์ด้วยแสงสุทธิ อัตราการคายน้ำ ค่าชักนำการเปิด-ปิดปากใบ สภาวะเครียดจากการขาดน้ำ การเปลี่ยนแปลงในรอบวัน

Introduction

Sugarcane is one of the most important industrial crops in the world. It is mainly used to produce sugar or biofuels like ethanol for renewable energy. Sugarcane is a C4 plant that fixes CO₂ into oxaloacetate within mesophyll and bundle sheath cells. This product is converted into sucrose and translocated through the phloem to stalk parenchyma cells, where it is stored for industrial sugar production (Moore, 1995). The global population is experiencing climate change, which increases plant abiotic stresses such as heat and drought. These abiotic stresses severely impact plant growth and yield, with estimates suggesting that they reduce agricultural crop production by approximately 51–82% (Verma *et al.*, 2020). The Northeastern region (Isan) of Thailand is the largest area for rainfed sugarcane cultivation, characterized by unique environmental constraints such as high temperatures, drought, and low or erratic rainfall in sandy soils that severely affect sugarcane production. To address these issues, the KKFCRC has established the sugarcane breeding program to develop new high sugar yield varieties with high sugar content, or drought tolerance under rainfed area in sandy soil of ISAN region (Chanachai *et al.*, 2023). Introducing these new varieties will help reduce the genetic vulnerability and epidemic risks associated with the decade-long monoculture of the widely planted DOA KK3 variety (Buakom *et al.*, 2020). Photosynthesis involves two linked stages: light reaction in the thylakoid membrane and carbon fixation in the stroma. These processes produce

primary intermediates 3-PGA in C3 plant and oxaloacetate in C4 plant to synthesize carbohydrates from ATP and NADPH. Therefore, light is the crucial energy source for plant photosynthesis by three critical attributes: light intensity, light quality, and photoperiod (De Souza and Buckeridge, 2014; Santos and Diola, 2015; Wu *et al.*, 2025). Light is limited by the natural day-night cycle, which significantly affects daily photosynthesis, especially during stressful conditions like drought (De Souza *et al.* 2018). In sugarcane, drought stress directly affects photosynthesis by reducing stomatal conductance (g_s), the net photosynthesis (A), or the transpiration rate (E) (Buakom *et al.*, 2020; Graça *et al.*, 2010; Jaiphong *et al.*, 2016; Khonghintaisong *et al.*, 2017; Silva *et al.*, 2013), which consequently diminishes the accumulation of photosynthetic products. (Wang *et al.*, 2017). The highest carbon dioxide exchange rate and stomatal conductance occurred at midday (Du *et al.*, 2000). Sugarcane consistently showed significantly faster stomatal opening than closure over the diurnal variation (Suwannarut *et al.*, 2023). In the sugarcane cv. ‘GT 42’, the net photosynthesis, stomatal conductance, and transpiration rate began increasing after sunrise, reached maximum between 09:00 and 10:00, and decreased thereafter (Verma *et al.*, 2020). Moreover, Wang *et al.* (2022) indicated that the physiological characteristics of drought-resistant plants can not only be observed under water deficit stress but also be determined by the plant's recovery process after re-watering. Likewise, Aidar *et al.* (2014) reported that drought-tolerant plants can immediately restore physiological processes, resulting in increased photosynthesis rates and further

accelerated plant growth. According to photosynthesis fluctuates diurnally under field conditions in response to light, studies on the diurnal photosynthetic patterns of Thai sugarcane clones are limited. That knowledge represents one of characteristics for selecting promising clones within the rainfed breeding programs of the KKFCRC. Consequently, this study investigated diurnal photosynthetic parameters of mature Thai sugarcane clones under drought and rewatering to identify their adaptation strategies for survival under adverse environmental conditions.

Materials and Methods

Plant materials and experimental conditions

Sugarcane cv. DOA KK3 and two promising clones, KK07-599 and KK3/E09-1, were selected by the KKFCRC due to their high commercial cane sugar (C.C.S.) and sugar yield, respectively. (Chinnasaen *et al.*, 2021), were used in this study. The experiment was arranged in a split-plot design with three replications. Irrigation management served as the main plot factor, consisting of two treatments: well-watered and water-restricted conditions. The sugarcane genotypes were assigned to the subplots. In early January 2023, four 1.5-month-old sugarcane seedlings derived from bud cutting were planted per experimental unit in cement blocks (80 cm diameter × 100 cm height) containing 345 kg of Yasothorn soil collected from the KKFCRC. The initial chemical properties of the soil were as follows: pH 5.4, electrical conductivity (EC) of 0.033 dS cm⁻¹, 0.32% organic matter, 0.014% total N, 27.49 mg kg⁻¹ available P,

47.21 mg kg⁻¹ exchangeable K, and 280.53 mg kg⁻¹ exchangeable Ca. Chemical fertilizer grades 15-15-15 at rate of 312.5 kg ha⁻¹ was applied on planting date and at 60 DAPs. Drip irrigation was supplied according to the sugarcane water requirement calculated by the formula, $ET_{crop} = ET_o \times K_c$, where ET_{crop} is the crop water requirement (mm day⁻¹), ET_o is the reference crop water requirement (mm day⁻¹), and K_c is the crop water requirement coefficient for sugarcane (Paisanchaoen *et al.*, 2012). From 298 to 316 DAP, one group of sugarcane was subjected to 19 days of water deficit followed by re-watering at 317 DAP, while the other group remained well-watered throughout the experiment. Photosynthetic parameters, daily climate, and soil moisture content were measured. The experiment was conducted under natural environmental conditions in Northeast Thailand at the KKFCRC (16°28'55.6"N and 102° 49'35.3"E), Sila, Mueang Khon Kaen, Khon Kaen Province.

Meteorological data and soil moisture contents (SMC)

Daily meteorological data including relative humidity, total rainfall, and minimum and maximum temperatures were recorded by the KKFCRC's weather station, located 1.5 km from the experimental field, from 294 to 320 DAPs. Soil samples were collected in triplicate at depths of 0–20, 20–50, and 50–80 cm for each sugarcane genotype and irrigation management. Sampling was performed at 297, 304, 311, 316, and 320 DAPs to determine soil moisture content. The soil samples were weighed before and after drying

in a hot-air oven at 120°C for 72 hours, and the moisture content was calculated as per Khonghintaisong *et al.* (2017) as follows:

soil moisture content (%)

$$= \frac{[(\text{wet weight} - \text{dried weight})]}{\text{dried weight}} \times 100$$

Photosynthetic parameters

Data were collected from November to December 2023. Photosynthetic parameters were measured in the second or third youngest, fully expanded, healthy leaves with three replications per sugarcane genotypes in each irrigation management. Measurements were taken between 06:00 and 18:00 h at 2-hour intervals on sunny days at 297, 316, and 320 DAPs, corresponding to before water restriction (day 0), after 19 days of water restriction (day -19), and 3 days after re-watering (day +3), respectively. Photosynthetic parameters including net photosynthetic rate (A), internal CO₂ concentration (Ci), transpiration rate (E), and stomatal conductance (gs) were measured using an automated photosynthesis measurement system (LCpro T Advanced, ADC BioScientific Ltd., Hoddesdon, UK). The chamber light source was set at 1,500 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (Liu *et al.*, 2020), whereas the CO₂ concentration and temperature tracked ambient conditions in each collection date.

Statistical analysis

The differences of photosynthetic parameters between the average values of the water-restricted and well-watered treatments at the same variation times for each genotype were

compared using *p*-values of a *t*-test in a statistical software package.

Results

Meteorological data and soil moisture contents

Daily climate data during the experimental period are presented in Figure 1-A. Throughout the experiment, the average minimum and maximum temperatures were 20.5 and 31.7°C, respectively, while the average relative humidity was 68.6%, and the total rainfall was 0.4 mm. A previous study reported that the SMC at the permanent wilting point for sugarcane was 4.2% at a soil depth of 0–20 cm (Paisanchaoen *et al.*, 2012). Therefore, the SMC at three depth levels were monitored during the water-restricted period. After 19 days of water restriction, the average SMC at depths of 0–20, 20–50, and 50–80 cm decreased to 3.86, 4.68, and 3.82%, respectively, whereas the average SMC of the well-watered group were 7.62, 7.25, and 6.50% (Figures 1B–D). Since irrigation was scheduled every seven days, the SMC was lowest on day 316 and increased by day 320, three days after re-watering. To avoid causing irreversible damage to the sugarcane plants, the water restriction period was terminated after 19 days (on day 316), followed by three days of rehydration to observe sugarcane recovery from the drought stress. Moreover, on days 297, 316, and 320, the sunrise times in Khon Kaen Province were 06:09, 06:18, and 06:21 h, while the sunset times were 17:36, 17:34, and 17:34 h, respectively (The Thai Astronomical Society, 2023).

Net photosynthesis rate (A)

Prior to irrigation management on day 297, all three genotypes experienced an increase in net photosynthetic rates (A) from morning through midday. The net photosynthesis rates of all three genotypes peaked between 08:00 and 12:00 h at approximately $15 \mu\text{mol CO}_2\text{m}^{-2} \text{s}^{-1}$ before decreasing at noon (12:00 h). By evening (18:00 h), the net photosynthesis rates dropped to nearly zero (Figures 2A–C). On day 316, following 19 days of water restriction, all sugarcane genotypes in the water-restricted (drought) group exhibited significantly lower net photosynthesis rates than their well-watered counterparts. Notably, KK3/E09-1 showed a significant difference between 10:00 and 16:00 h. Furthermore, the net photosynthesis rates on this day showed a declining trend from noon through the evening, mirroring the pattern observed on day 297 (Figures 2D–F). After three days of rehydration (on day 320), almost all sugarcane genotypes showed no significant differences in net photosynthesis rates between the well-watered and water-restricted groups. The net photosynthesis rates gradually increased from early morning to midday, peaking at 08:00 h at approximately $10 \mu\text{mol CO}_2\text{m}^{-2} \text{s}^{-1}$ before slowly decreasing to nearly zero by dusk (18:00 h) (Figure 2, G–I).

Internal CO₂ concentration (Ci)

The diurnal changes in internal CO₂ concentration (Ci) for all sugarcane genotypes on day 297 (before water restriction) revealed a consistent pattern, peaking at approximately $400 \mu\text{molmol}^{-1}$ in the early morning (06:00 h). This

was followed by a rapid decrease to $100\text{--}200 \mu\text{mol mol}^{-1}$ during the daytime (08:00 to 16:00 h) and a subsequent increase at 18:00 h (Figures 3A–C). On day 316, after 19 days of water restriction, all sugarcane plants exhibited a similar internal CO₂ concentration pattern: high at dawn (06:00 h), dropping during the day, and rising again in the evening (18:00 h). Generally, the internal CO₂ concentration values under the water-restricted were higher than those under the well-watered, particularly between 10:00 and 18:00 h (Figures 3D–F). On day 320, after three days of rehydration, the diurnal changes in internal CO₂ concentration for all sugarcane genotypes followed a pattern similar to that observed on day 297. No significant differences were found between the well-watered and rehydrated groups within the same varieties, except for KK3/E09-1, which exhibited a significantly higher internal CO₂ concentration in the well-watered group than in the rehydrated group at 10:00 h (Figure 3, G–I).

Transpiration rate (E)

On day 297, prior to irrigation management, the transpiration rates (E) of all sugarcane genotypes gradually increased from $0.58\text{--}1.37 \text{ mmol m}^{-2} \text{s}^{-1}$ at 06:00 h to a midday peak, with DOA KK3 reaching $2.63 \text{ mmol m}^{-2} \text{s}^{-1}$. Subsequently, the transpiration rates declined toward zero, with an average value of $0.20 \text{ mmol m}^{-2} \text{s}^{-1}$ by evening (Figures 4A–C). On day 316, after 19 days of water restriction, all sugarcane genotypes across both irrigation practices showed an increase in transpiration rates from 06:00 to 08:00 h. Thereafter, the water-restricted groups experienced

a continuous decline until 18:00 h in KK3/E09-1, or showed a slight secondary increase between 14:00 and 16:00 h before dropping to nearly zero at 18:00 h. Overall, the well-watered group maintained significantly higher transpiration rates than the water-restricted group, particularly for KK07-599 and KK3/E09-1. The highest transpiration rate was observed in the well-watered group of KK07-599, reaching $2.08 \text{ mmol m}^{-2} \text{ s}^{-1}$ at 12:00 h (Figures 4D–F). Following three days of rehydration (on day 320), the transpiration rates of all sugarcane genotypes were nearly zero at 06:00 h. The rates increased throughout the day, with the well-watered DOA KK3 group exhibiting the highest transpiration rate at 14:00 h, before returning to nearly zero by 18:00 h (Figures 4G–I).

Stomatal conductance (g_s)

On day 297, prior to water restriction, the stomatal conductance (g_s) of KK07-599 and KK3/E09-1 increased slowly from approximately $0.10 \text{ mmol m}^{-2} \text{ s}^{-1}$ in the early morning (06:00 h) before declining to nearly zero by evening (18:00 h).

Meanwhile, the stomatal conductance of DOA KK3 increased slightly from early morning into the daytime, peaking at around $0.10 \text{ mmol m}^{-2} \text{ s}^{-1}$, before decreasing at 14:00 h and reaching nearly zero at 18:00 h (Figures 5A–C). After 19 days of water restriction (drought condition), the stomatal conductance of all genotypes increased from 06:00 to 08:00 h across both irrigation managements. The highest stomatal conductance values were recorded at 08:00 h, ranging from $0.05\text{--}0.06 \text{ mmol m}^{-2} \text{ s}^{-1}$ for KK07-599, $0.06\text{--}0.09 \text{ mmol m}^{-2} \text{ s}^{-1}$ for KK3/E09-1, and $0.04\text{--}0.07 \text{ mmol m}^{-2} \text{ s}^{-1}$ for DOA KK3, followed by complete stomatal closure by 18:00 h. The water-restricted group consistently maintained lower stomatal conductance values than the well-watered group across all genotypes, with significant differences observed at 10:00 and 14:00 h, particularly in KK3/E09-1 (Figures 5D–F). Following three days of rehydration (on day 320), the stomatal conductance of all genotypes was nearly zero at 06:00 h, increased during the daytime, and declined after 16:00 h, leading to stomatal closure by 18:00 h (Figures 5G–I).

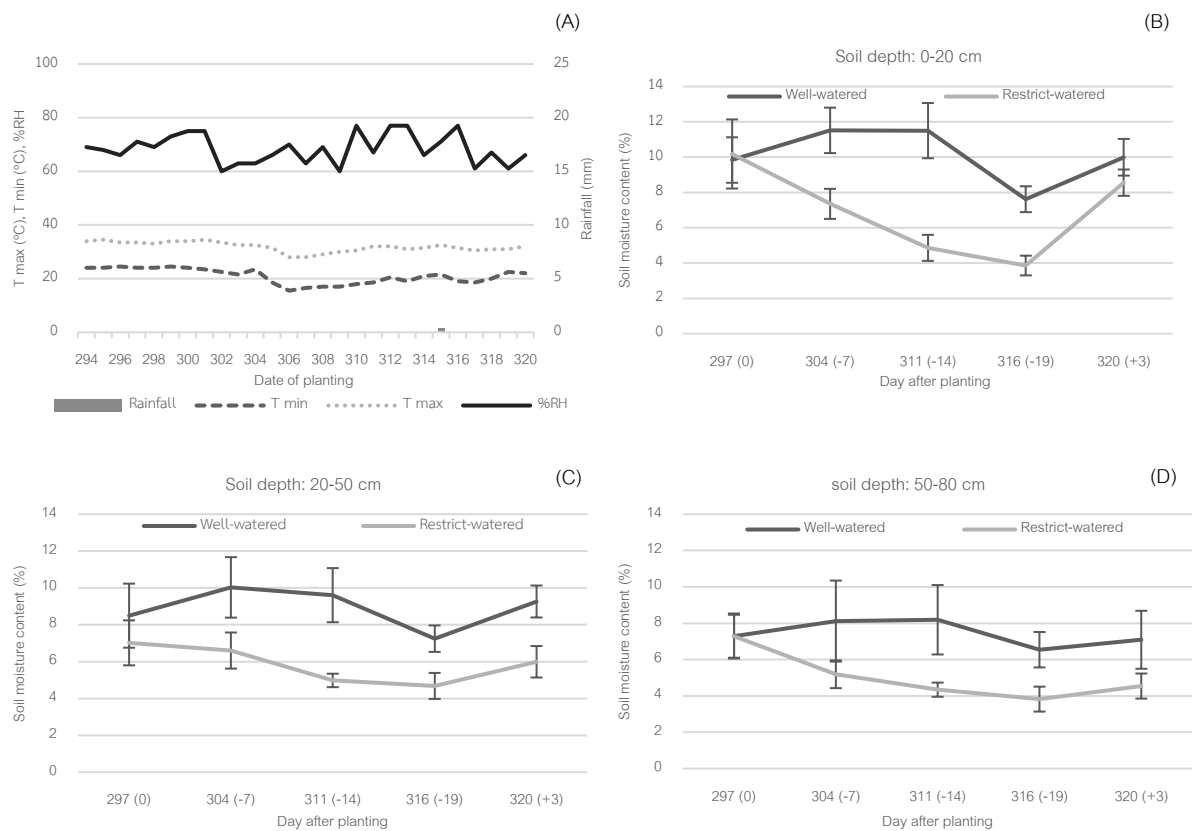


Figure 1 Daily climate (A) and soil moisture content at various soil depths of 0–20 cm (B), 20–50 cm (C), and 50–80 cm (D), (data are mean±SE) during conducting the experiment at KKFCRC, Khon Kaen Province, on 5 to 27 November, 2023. The water-restricted period was at 298–316 days after planting

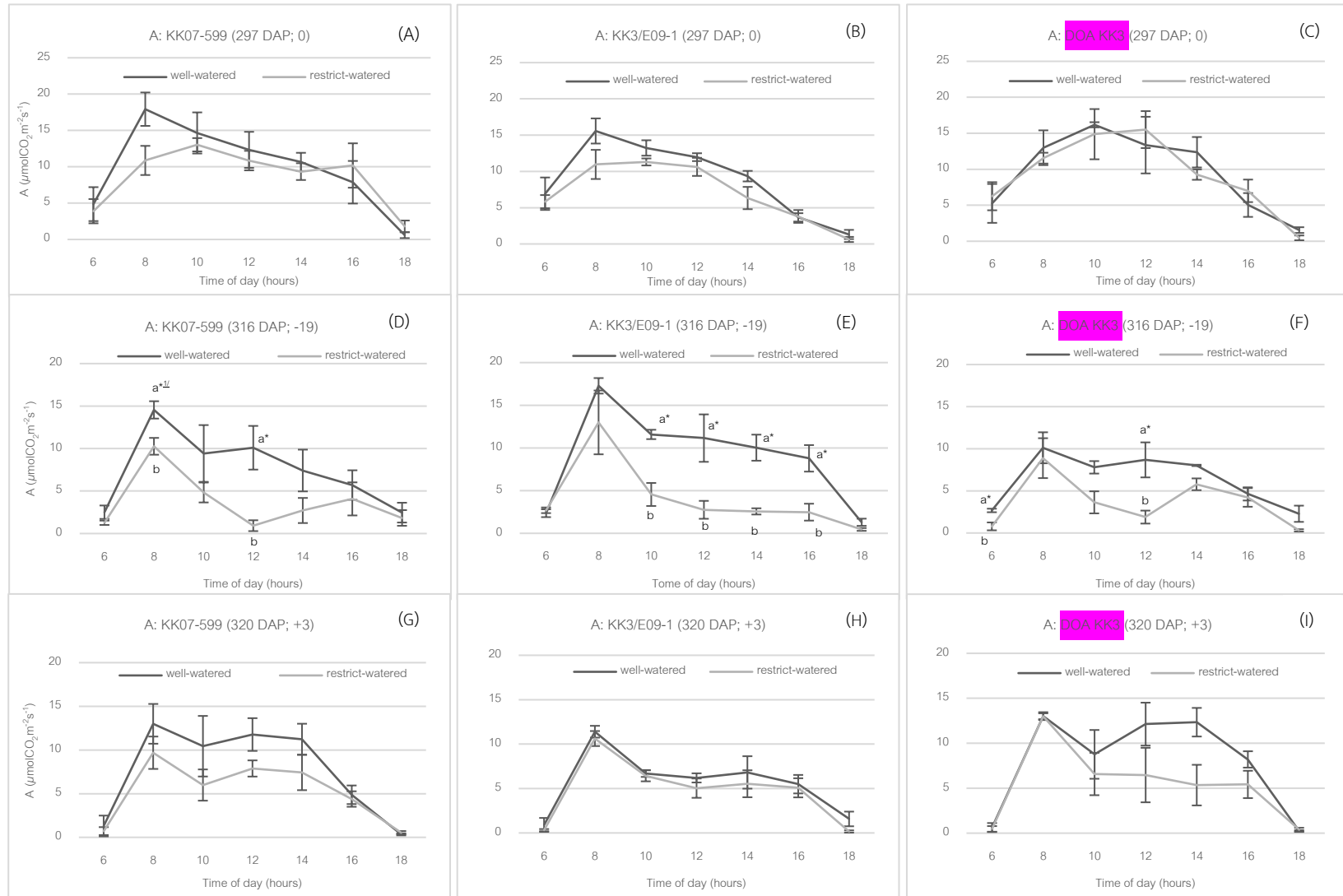


Figure 2 Diurnal net photosynthesis rate (A) of KK07-599, KK3/E09-1, and DOA KK3 on day 297 or before water restriction (A–C), on day 316 or after 19 days of water restriction (D–F), on day 320 or three days after rehydration (G–I). (¹⁴Mean followed by different letters in each day are significantly different by *p*-values of a *t*-test, * = Significant at *p* < 0.05, ** = Significant at *p* < 0.01.)

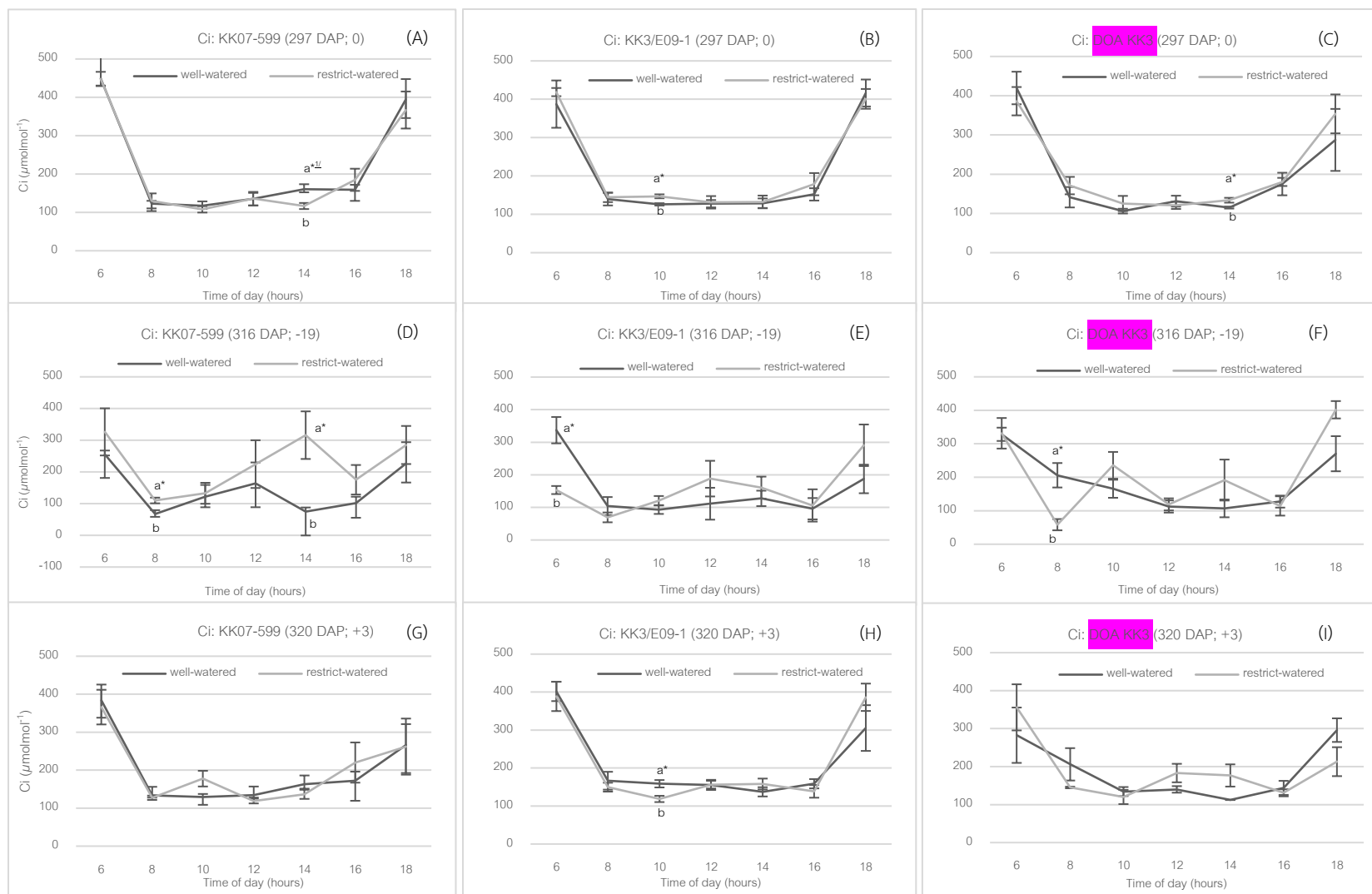


Figure 3 Diurnal Internal CO₂ concentration (Ci) of KK07-599, KK3/E09-1, and DOA KK3 on day 297 or before water restriction (A–C), on day 316 or after 19 days of water restriction (D–F), on day 320 or three days after rehydration (G–I). (¹⁴Mean followed by different letters in each day are significantly different by *p*-values of a *t*-test, *=Significant at *p*<0.05, **=Significant at *p*<0.01.)

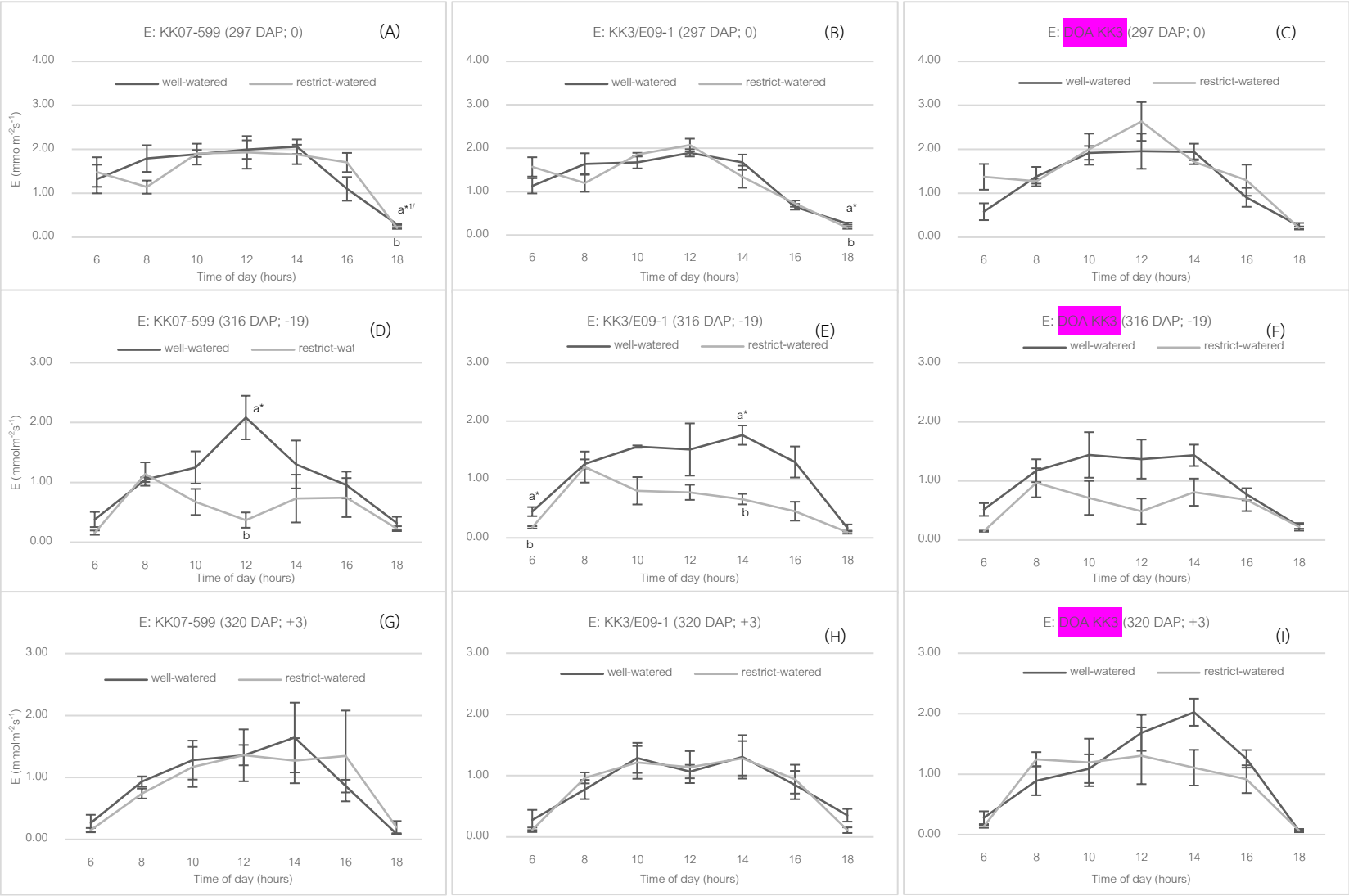


Figure 4 Diurnal Transpiration rate (E) of KK07-599, KK3/E09-1, and DOA KK3 on day 297 or before water restriction (A–C), on day 316 or after 19 days of water restriction (D–F), on day 320 or three days after rehydration (G–I). (¹⁴Mean followed by different letters in each day are significantly different by *p*-values of a *t*-test, *=Significant at $p < 0.05$, **=Significant at $p < 0.01$.)

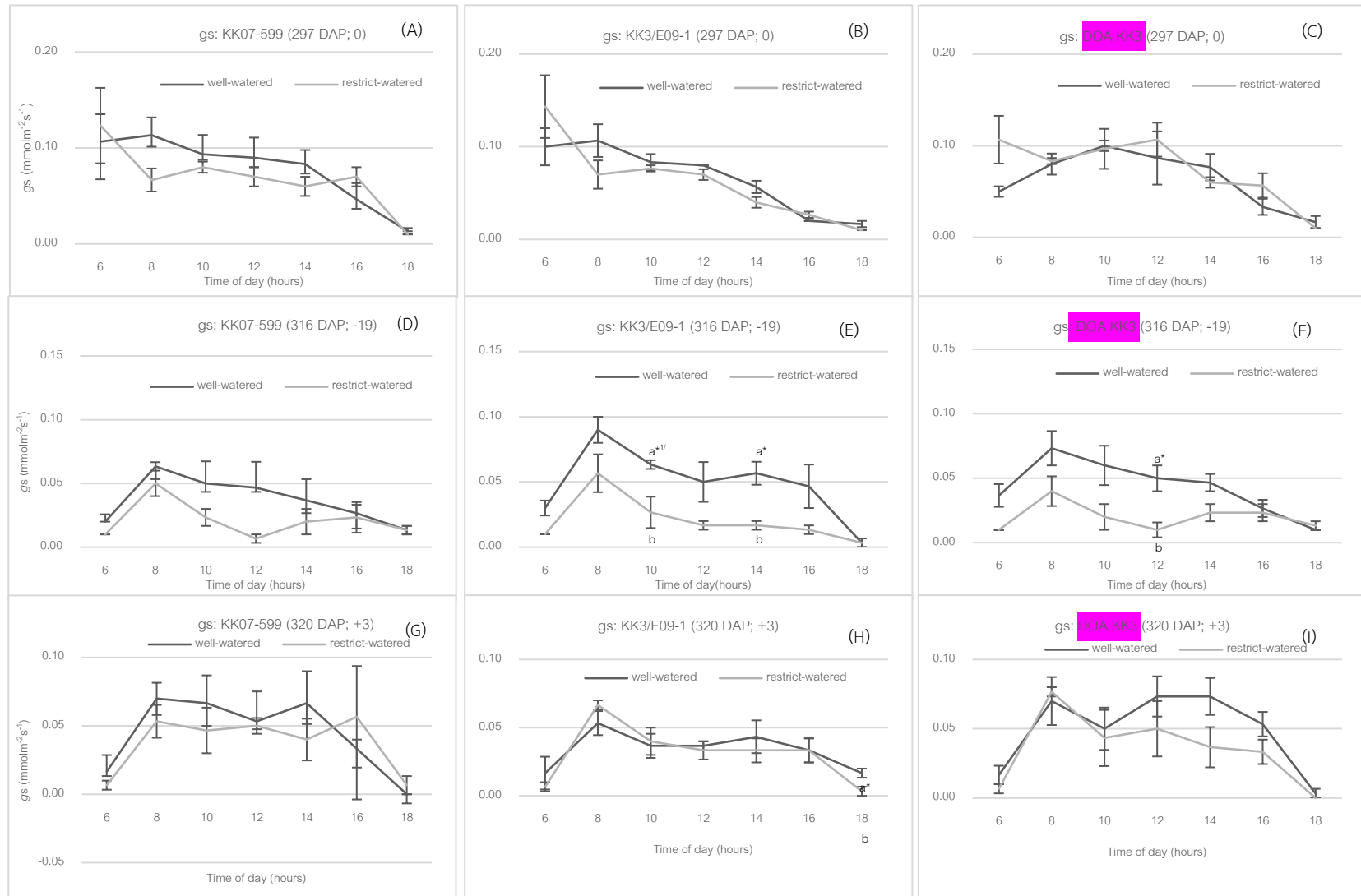


Figure 5 Diurnal Stomatal conductance (gs) of KK07-599, KK3/E09-1, and DOA KK3 on day 297 or before water restriction (A–C), on day 316 or after 19 days of water restriction (D–F), on day 320 or three days after rehydration (G–I). (¹⁴Mean followed by different letters in each day are significantly different by *p*-values of a *t*-test, * = Significant at *p* < 0.05, ** = Significant at *p* < 0.01.)

Discussion

Photosynthesis primarily produces oxygen and sugar, providing organisms with essential carbon and energy. Because sugarcane directly stores sugar as sucrose within stalk parenchymatous cells, improving sucrose yield requires breeding programs to develop cultivars that can both accumulate sugar rapidly and maintain a high accumulation content throughout the growth cycle. This includes the mature growth stage (days 286–375), during which the continuous transport of sucrose to the stalk is maintained (Paisancharoen *et al.*, 2012). Photosynthesis is regulated by the photoperiod, or light exposure throughout the day-night cycle, which drives daily fluctuations in photosynthetic activity. Furthermore, other environmental factors influence photosynthesis particularly water availability, which fluctuates with climate change and leads to water deficit or drought stress. Additionally, the ability to tolerate water deficit varies across different sugarcane genotypes. Gomathi *et al.* (2011) reported that drought-tolerant sugarcane genotypes (Co99004 and Co99008) transpired less water and maintained relatively higher photosynthesis rates, resulting in significant improvements in growth attributes.

On day 297, all experimental units received irrigation based on crop water requirements. The initial net photosynthesis rates at 06:00 h for all sugarcane genotypes were approximately $5.00 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, while the transpiration rates and stomatal conductance values were above 1.00 and $0.10 \text{ mmol m}^{-2} \text{ s}^{-1}$, respectively. These findings

suggest that sugarcane is capable of performing photosynthesis as soon as it receives suitable light, even during the early morning hours. This high initial activity may be attributed to the sufficient soil moisture content present in all irrigated managements. Water serves as the electron donor for the electron transport chain (ETC) from photosystem II (PSII) to PSI, ultimately producing NADPH and ATP to drive the carbon reduction cycle. In this study, although the sugarcane plants were exposed to a constant light intensity of $1,500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ during the measurements, diurnal variations in photosynthetic parameters fluctuated across all genotypes. The net photosynthesis rate, transpiration rate, and stomatal conductance tended to peak in the morning or midday (08:00 to 12:00 h) and declined thereafter, whereas the internal CO_2 concentration was high at dawn (06:00 h) and dusk (18:00 h), remaining stable between 08:00 and 16:00 h.

Generally, plants possess an endogenous timer known as the circadian clock, which regulates metabolic processes in accordance with the diurnal patterns of gene expression, including those in photosynthetic and related systems. Stomatal conductance typically increase in the morning and decrease in the afternoon, whereas the net photosynthesis rate peaks at midday. However, the exact timing of these peaks varied in a species-specific manner (De Dios, 2017). Moreover, stomatal conductance was often lower in the afternoon than in the morning because stomatal closure is more sensitive to abscisic acid (ABA) during the afternoon due to

interactions with clock-component genes (Seo and Mas, 2015). In accordance with other reports, Verma *et al.* (2020) reported that diurnal climatic variables such as photosynthetic photon flux density (PPFD) and vapor pressure deficit (VPD) were low in the morning, elevated during daylight hours (particularly at 15:00 h), and declined by evening. Conversely, relative humidity (RH) and ambient CO₂ concentration were high at dawn and dusk but decreased during the daytime. Likewise, Du *et al.* (2000) observed that the diurnal patterns of photosynthetically active radiation (PAR), carbon dioxide exchange rate (CER), and stomatal conductance peaked at midday, indicating that photosynthesis in sugarcane was almost completely regulated by sunlight.

After 19 days of water restriction, the water-restricted sugarcane groups exhibited distinctly lower net photosynthetic rates, transpiration rates, and stomatal conductance than the well-watered group across all genotypes. This reduction occurs because plants close their stomata during drought to conserve water, which subsequently limits photosynthetic activity (Chaves *et al.*, 2002; Gupta *et al.*, 1989). Consistently, Silva *et al.* (2013) found highly significant positive correlations under water deficit between net photosynthesis and stomatal conductance ($r=0.96$, $p<0.01$), as well as between net photosynthesis and transpiration ($r=0.93$, $p<0.01$). Similarly, Leanasawat *et al.* (2021) reported that the net photosynthesis rate, transpiration rate, and stomatal conductance of sugarcane genotypes under soil field water capacity (FWC)

exceeded those under 50% field capacity (FC) or rainfed conditions. Specifically, for genotype DOA KK3, the net photosynthetic rate was 36.76 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ under FWC, but dropped to 35.35 and 25.23 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ under 50% FC and rainfed conditions, respectively; corresponding transpiration rates were 3.47, 2.13, and 2.82 $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$. This result concerted with Gomathi *et al.* (2011), who reported that the net photosynthesis rate of sugarcane under well-watered conditions exceeded that under drought, at 18.49 and 12.21 $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively; corresponding transpiration rates were 1.78 and 1.51 $\text{mmol m}^{-2} \text{ s}^{-1}$. Notably, sugarcane genotypes capable of maintaining their photosynthetic potential under water deficit are classified as drought-tolerant (Ribeiro *et al.*, 2013). Carbon fixation, a key photosynthetic reaction, involves carbon dioxide fixation by ribulose-1,5-bisphosphate (RuBP) catalyzed by ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) to produce carbohydrates (Osborne and Sack, 2012). In this study, the diurnal internal CO₂ concentration was higher under water deficit than under well-watered conditions. This could be attributed to drought-induced water limitation, which disrupts the electron transport chain (ETC) in (PSII), given that water serves as the primary electron donor for PSII via the oxidation process (Lawlor and Cornic, 2002). Similarly, Silva *et al.* (2007) concluded that drought stress reduced the photosynthetic efficiency of PSII, thereby decreasing the production of chemical energy (ATP and NADPH). Consequently, this insufficient energy supply restricts the regeneration phase of the carbon reduction

cycle, limiting RuBP availability for carbon dioxide fixation.

Three days after rehydration (on day 320), the diurnal patterns of stomatal conductance in most water-restricted groups resembled those of the well-watered group. Similarly, net photosynthesis rate, transpiration rate, and internal CO₂ concentration showed no significant differences compared to the well-watered control. Following rehydration, sugarcane uptake water to restore cell turgor, which triggers stomatal opening and maintains transpiration rate for gas exchange (Graça *et al.*, 2010; Dos Santos *et al.*, 2014). Sugarcane cv. DOA KK3 has been widely reported as a cultivar capable of rapid drought recovery upon rehydration (Buakom *et al.*, 2020; Namwongsa *et al.*, 2019). In this study, the promising clone KK07-599 and DOA KK3 exhibited robust recovery with high stomatal conductance values, whereas KK3/E09-1 maintained low stomatal conductance, transpiration rate, and net photosynthetic rate under both well-watered and rehydrated conditions. Graça *et al.* (2010) reported that sugarcane cultivars varied in their photosynthetic parameters under water deficit, with tolerant cultivars (SP83-2847 and CTC15) maintaining higher PSII efficiency than the sensitive cultivar (SP86-155). At 8 days after rehydration, CTC15 and SP86-155 showed slight increases in photosynthesis rates; by day 10, all cultivars presented high photosynthesis rates compared to the water-deficit condition, with SP83-2847 demonstrating the best overall performance. Similarly, Dos Santos *et al.* (2014) found that RB92579 and SP81-3250 showed high

stomatal sensitivity and reduced PSII under drought. Beyond genetic variation, the recovery duration (days after rehydration) also plays a critical role in restoring photosynthetic activity. Photosynthesis can recover immediately upon re-watering (Dinh *et al.*, 2019); for instance, sugarcane cv. RB962962 subjected to an 8-day water restriction rapidly restored its photosynthetic activity by the second day of rehydration (Medeiros *et al.*, 2013)."

Conclusions

Although this study did not assess all drought-tolerant sugarcane genotypes, survival under water deficit was clearly linked to stomatal closure as an adaptive mechanism against water shortage. For all genotypes, the net photosynthesis rate, transpiration rate, and stomatal conductance increased from morning to midday and subsequently declined toward evening. Conversely, internal CO₂ concentrations peaked at dawn and dusk, remaining low and stable during the daytime. Under drought conditions, sugarcane exhibited reduced photosynthetic parameters but elevated internal CO₂ concentrations compared to the well-watered condition; however, after rehydration for 3 days, these parameters returned to levels similar to those of well-watered plants. Furthermore, understanding these diurnal variations in photosynthetic parameters provides strategic directions for sugarcane cultivation, such as optimizing irrigation scheduling during peak stomatal opening in the morning to maximize transpiration and photosynthesis. Nevertheless, further research is required to elucidate the direct

relationship between photosynthetic efficiency and sugar accumulation to enhance sugar yield and establish robust selection criteria for sugarcane breeding programs.

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