



## **EVALUATION OF SUGARCANE ELITE CLONES THROUGH PHYSIOLOGICAL RESPONSES AND YIELD RELATED TRAITS UNDER EARLY RAINFED DROUGHT STRESS CONDITIONS**

**P. SONGSRI<sup>1,2,\*</sup>, J. NATA<sup>1</sup>, N. BOOTPROM<sup>2</sup> and N. JONGRUNGKLANG<sup>1,2</sup>**

<sup>1</sup> Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand

<sup>2</sup> Northeast Thailand Cane and Sugar Research Center (NECS), Khon Kaen University, Khon Kaen, Thailand

\*Corresponding author's email: patcharinso@kku.ac.th

Email addresses of coauthors: raylovedad@gmail.com, namaoy\_bio@hotmail.com,  
 nuntawootjrk@gmail.com

### **SUMMARY**

Sugarcane (*Saccharum* sp.) is one of the most important sugar producing crops, and plays a key role in ethanol production in most tropical and subtropical countries. Selection of sugarcane varieties has traditionally been based on yield and agronomic traits; however, the use of physiological traits in sugarcane selection may increase selection efficiency. The objective of this study was to investigate the effects of physiological responses on yield and yield components in sugarcane under rainfed conditions. Nineteen elite sugarcane clones were evaluated in a randomized complete block design with three replications, from January, 2015 to January, 2016; at the Agronomy Research Station, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand. The plots consisted of four rows, six meters in length, and spacing of 1.5 m x 0.5 m. Data were recorded for chlorophyll fluorescence, SPAD chlorophyll meter reading (SCMR), relative water content (RWC), stomatal conductance at 90 and 180 days after planting (DAP), stalk height, increased stalk height rate, number of tillers/stool, number of stalks/stool at 90, 180, and 270 days after planting (DAP); stalk diameter, stalk length, number of stalks/stool, single stalk weight, millable cane, cane yield, and total soluble solids at 12 months after planting (MAP). KK3 proved to be a superior clone for agronomic traits; such as cane yield, millable cane, stalk diameter, stalk length, total soluble solids; and was also associated with several physiological traits; such as high photosynthesis efficiency, SCMR, and stomatal conductance. Other comparative clones; such as KK07-037, MPT02-458, CSB07-79, K88-92, KCU99-01, and TBy28-0941 had similarly high cane yields; whereas CSB07-219, MPT02-458, KCU99-02, KCU99-03, TP06-419, and UT13 had higher total soluble solids.

**Key words:** Cane yield, leaf color, photosynthesis efficiency, SPAD chlorophyll meter reading (SCMR), stomatal conductance, relative water content (RWC), sugarcane breeding

**Key findings:** Under early drought stress conditions, sugarcane clones capable of maintaining higher yields demonstrated superior adaptation during drought periods, and higher growth rates in recovery periods. The physiological traits; SCMR, Fv/Fm, RWC, and stomatal conductance were important factors contributing to the sugarcane biomass yields. Under early drought stress conditions, sugarcane clones capable of maintaining higher yields demonstrated superior adaptation during drought periods, and higher growth rates in recovery periods. The physiological traits; SCMR, Fv/Fm, RWC, and stomatal conductance were important factors contributing to the sugarcane biomass yields.

Manuscript received: March 15, 2019; Decision on manuscript: June 17, 2019; Accepted: September 11, 2019.  
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Communicating Editor: Dr. Bambang S. Purwoko

## INTRODUCTION

Sugarcane (*Saccharum* spp.) is one of the most important industrial crops in the world. The leading sugarcane producing countries of the world are Brazil, India, China, and Thailand. Cane sugar contributes roughly 80% of the total sugar produced in the world. The remaining 20% production of sugar is contributed by beet sugar and other sources, such as hydrolyzed starch products (Chidambaram and Sivasubramaniam, 2017).

Drought is a recurring problem of sugarcane production, which relies solely on rainfall. Generally, sugarcane is produced in areas with insufficient amounts of good quality water available through irrigation. Most sugarcane production areas in Thailand are located in rain-fed conditions (Laclau and Laclau, 2009), and drought usually appears during growing season especially early season drought during December to April (Khonghintaisong *et al.*, 2018). Most sugarcane production areas do not receive an adequate supply of

water throughout the growth period, resulting in yield reduction (Inman-Bamber, 2004; Silva *et al.*, 2008; De-Silva and De-Costa, 2009; Ishaq and Olaoye, 2009; Cha-Um *et al.*, 2012). Drought is a major factor affecting growth and yield of sugarcane, and yield reduction could be as high as 60% sugarcane (Robertson *et al.* 1999). Selection of sugarcane varieties for drought tolerance will sustainably solve this problem.

The development of drought tolerant cultivars has been one of the most important improvements in crop management; which includes sugarcane breeding (Inman-Bamber and Smith, 2005), and the identification of important physiological mechanisms underlying drought resistance, which are necessary in determining selection criteria (Smit and Singels, 2006). As sugarcane varieties respond differently to drought stress, the identification of drought resistant genotypes and the development of drought resistant varieties are essential, particularly in

drought-prone areas (Silva *et al.*, 2011).

Rong-hua *et al.* (2006) reported that the indirect and more rapid methods of measuring photosynthetic activity in barley; such as the chlorophyll fluorescence technique, the maximum photochemical efficiency of photosystem II-PSII (assessed via the variable-to-maximum chlorophyll to fluorescence ratio,  $F_v/F_m$ ), and the estimated chlorophyll content (SPAD unit) can be as effective as the more time-consuming gas exchange techniques in revealing differences in drought tolerance among susceptible genotypes. The relationship between drought tolerance and chlorophyll fluorescence using a portable fluorometer has been well established in sugarcane (Luo *et al.*, 2004; Molinari *et al.*, 2007; Silva *et al.*, 2007). Other physiological parameters, such as relative water content (RWC) are also very responsive to water stress, and have been shown to be well correlated with drought tolerance in barley and wheat (Jamaux *et al.*, 1997; Altinkut *et al.*, 2001; Colom and Vazzana, 2003). Visual assessment of the agronomic performance and the overall varietal response to drought remains the common method of selection for drought tolerance in sugarcane (Wagih *et al.*, 2001).

Most studies conducted to date have based their selection of sugarcane genotypes on the agronomic traits of a minimal number of available genotypes. The objective of this study was to investigate the effects of physiological responses (relative water content, chlorophyll fluorescence, SPAD chlorophyll meter reading and stomatal conductance) and growth traits on yield and yield

components of 19 sugarcane clones under rainfed conditions.

## MATERIALS AND METHODS

### Plant material

Nineteen elite sugarcane clones (TBy28-1211, TBy28-0941, Kps01-12, KK3, MPT02-458, MPT05-187, K88-92, UT12, UT13, CSB07-79, CSB07-219, TP06-419, KK06-501, KKU99-01, KKU99-02, KKU99-03, KKU99-06, Q229, and KK07-037) were evaluated in a randomized complete block design (RCBD) with three replications from January, 2015 to January, 2016 at the Faculty of Agriculture, Khon Kaen University, Thailand. The KK3 is the most popular variety in Thailand and it was used as a high yielding standard check. UT12 was used as a susceptible check in this study. The sugarcane clones were planted in the 4-row plots, six meters in length, and spaced 1.5 m × 0.5 m. Basal fertilizers at the rates of 312.5 kg N, 312.5 kg P, and 312.5 kg K ha<sup>-1</sup> were applied to each pot immediately after planting; and top dressing fertilizers were applied at the rates of 312.5 kg N, 312.5 kg P, and 312.5 kg K ha<sup>-1</sup> four months after planting. Weeds, insects, and diseases were controlled for optimum crop growth.

### Data collection

#### *Soil moisture content and meteorological conditions*

Soil moisture content was measured at 90, 180, and 270 days after planting (DAP) using the micro-auger method at 15-30 and 30-45 cm below the soil surface for irrigation purposes. Rainfall, maximum temperature,

minimum temperature, and relative humidity were recorded daily throughout the experimental period at a weather station located 100 meters from the experimental field.

#### *Growth traits*

Number of tillers/stool and stalk height were recorded in the drought stress period (90 DAP), recovery period (180 DAP), and full-growth period (270 DAP). Number of tillers/stool was counted within each plot for the perfect tiller. Stalk heights were recorded from the ground to the last-exposed dewlap. Increases in stalk height/day were calculated from the following equation:

$$\text{Height increasing rate (cm/day)} = \frac{(H_2 - H_1)}{(T_2 - T_1)}$$

H = stalk high (cm), at Date 1 and Date 2

T = days after planting, at Date 1 and Date 2

#### *Physiological traits*

Stomatal conductance, chlorophyll fluorescence, SPAD chlorophyll meter reading (SCMR), and relative water content (RWC) were recorded from the upper two-thirds of the fully expanded leaf from the top of the main stem at 90 and 180 DAP. Stomatal conductance was measured using a steady-state porometer (model AP4, Delta-T Devices, Cambridge, UK) daily, between 10.00 AM and noon. Chlorophyll fluorescence was measured using a chlorophyll fluorescence meter (PAM-2000, Heinz Walz GmbH, Germany) with the method described by Maxwell and Johnson, (2000); in order to quantify the levels for drought-induced photo-

inhibition. The measured leaves were dark-adapted for 15 minutes using leaf clips (FL-DC, Opti-Science) before the chlorophyll fluorescence was measured. SCMR was determined using a SPAD-502 chlorophyll meter (Minolta SPAD-502 m, Tokyo, Japan) daily, between 9:00 AM and noon. RWC was calculated from the following equation with minor modifications (Matin *et al.*, 1989):

$$\text{RWC} = [(W_f - W_d)/(W_t - W_d)] \times 100$$

Leaf disc fresh weight (W<sub>f</sub>) was determined within two hours of excision. The turgid weight (W<sub>t</sub>) was obtained after hydration in deionized water for 24 hours, in darkness, at room temperature. Leaf discs were quickly blotted and oven-dried for 72 hours at 80°C before recording the dry weight (W<sub>d</sub>).

#### *Yield and yield component traits*

Data were recorded for stalk diameter, stalk length, number of stalks/stool, single stalk weight, millable cane, yield, and total soluble solids at harvest; 12 months after planting. The number of millable stalks was counted within each plot. In the determination of the stalk length, a measuring tape was used to measure a sample of six stalks. A vernier caliper was used to measure the diameter of the same six stalks, in which the reading region was defined as one-third of the stalk length (from the base to the top). Then, the six stalks were weighed, and the mean weight was obtained. The cane yield (t/ha) was calculated from the weight of all millable canes per plot within the harvest area. Lastly, the total soluble solid was measured by digital brix refractometer (ATAGO PAL1).

## Statistical analysis

The data for cane yield, yield components, and physiological traits were analyzed statistically according to a randomized complete block design (Gomez and Gomez, 1984). Analysis of variance was performed using mstat-C software, and Duncan's New Multiple Range Test (DMRT) at  $P = 0.05$  probability level was used to compare the mean differences. Pearson's simple correlation was calculated to determine the relationships among traits by using Statistix 10 software package.

## RESULTS

### Meteorological conditions and soil moisture content

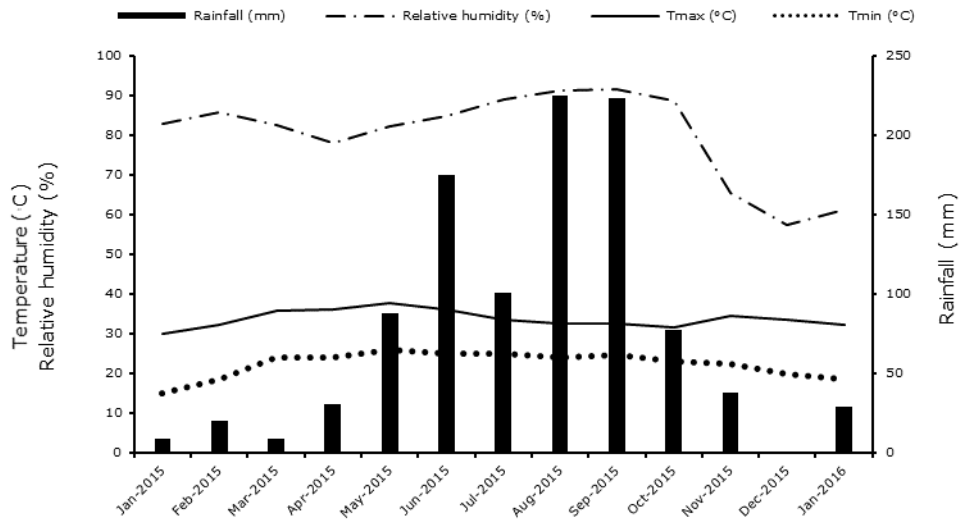
Maximum temperature, minimum temperature, rainfall, and relative humidity were recorded at a weather station located 100 meters from the experiment area in the growing seasons of January 2015 to January 2016, shown in Figure 1. Minimum air temperatures ranged from 15.2 to 26.1°C, and maximum temperatures ranged from 30.0 to 38.0°C during the growing season. The experiment received rainfall in most months, except in December 2015. August 2015 had the highest rainfall of 225 mm. The highest relative humidity (91.7%) was recorded in September 2015, and the lowest relative humidity (57.5%) was recorded in December 2015. In this study, drought stress caused by low amount of rainfall during January 2015 to April 2015.

Soil moisture contents in the upper soil layer (0-30 cm) were higher than the soil moisture contents in the lower soil layer (30-45 cm) evaluated

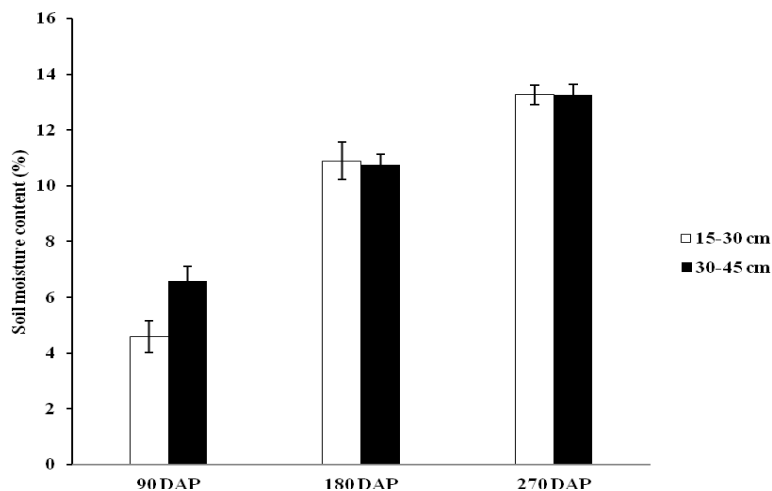
at 90 DAP. Soil moisture contents in the lower and upper soil layers were similar at 180 and 270 DAP, whereas soil moisture content in the upper soil layer was higher than the lower soil layer at 180 and 270 DAP (Figure 2).

### Cane yield and yield components

Variations among the 19 clones of sugarcane were evaluated for stalk diameter, stalk length, number of stalks/stool, single stalk weight, millable cane, yield, total soluble solids at harvest, stalk height, increased stalk height rate, number of tillers/stool, and number of stalks/stool in the drought stress period (90 DAP), recovery period (180 DAP), and full growth period (270 DAP). Sugarcane clones were significantly different ( $P \leq 0.01$  or  $P \leq 0.05$ ) for stalk diameter, stalk length, number of stalks/stool, single stalk weight, millable cane, yield, total soluble solids, stalk height, increased stalk height rate in the drought stress period (90 DAP), recovery period (180 DAP), and full growth period (270 DAP); number of tiller/stool at drought stress period (90 DAP), and number of stalks/stool in the recovery period (180 DAP) and full growth period (270 DAP) (Tables 1, 2, 3, and 4). In this study, MPT02-458, KK07-037, KK3, CSB07-79, K88-92, KKU99-01, and TBy28-0941 produced high cane yields (82.75 to 72.00 t/ha). KK3 and MPT02-458 also yielded high levels of total soluble solids. KK3 had high stalk diameter, stalk length, stalk number/stool, millable cane, and number of tillers/stool in the drought stress period (90 DAP). K88-92 and MPT02-458 had high stalk length. CSB07-79 and TBy28-0941 demonstrated high stalk length, single stalk weight, and number of



**Figure 1.** Rainfall (mm), maximum temperature (°C), minimum temperature (°C), and relative humidity (%) during the experiment period.



**Figure 2.** Soil moisture content in the upper soil layer (15-30 cm) and lower soil layer (30-45 cm) recorded at 90, 180, and 270 DAP.

tillers/stool in the drought stress period (90 DAP). KK07-037 had high stalk length, stalk number/stool, and millable cane. K KU99-01 had high stalk diameter, and number of tillers/stool in the drought stress period (90 DAP); whereas CSB07-219, TP06-419, K KU99-03, K KU99-02, and UT13 had high total soluble solids.

Most high yield groups including MPT02-458, KK07-037, KK3, K88-92, and K KU99-01 had low growth rates in response to drought, and high recovery of increased stalk height rate; whereas CSB07-79 and TBy28-0941 had high increased stalk height rates.

**Table 1.** Yield, millable cane, and total soluble solids of 19 sugarcane clones at harvest.

Clones	Yield (t ha <sup>-1</sup> )	Millable cane (stalk ha <sup>-1</sup> )	Total soluble solids (Brix°)
MPT02-458	82.75 a	62,500 bcd	22.03 a-d
KK07-037	81.83 a	71,667 a	17.07 i
KK3	77.67 ab	68,333 abc	22.83 ab
CSB07-79	74.33 abc	55,833 d-g	18.88 h
K88-92	74.17 abc	55,833 d-g	20.33 fg
KKU99-01	72.83 a-d	57,500 def	20.74 ef
TBy28-0941	72.00 a-e	56,667 d-g	19.29 gh
CSB07-219	67.50 b-f	48,333 hij	23.11 a
MPT05-187	63.67 b-g	57,500 def	20.87 ef
TBy28-1211	62.67 c-h	54,167 e-h	21.42 def
Q229	60.63 c-h	59,167 de	21.77 b-e
Kps01-12	59.33 d-h	61,667 cd	21.34 def
TP06-419	59.00 d-h	50,000 g-j	22.57 abc
KKU99-03	58.17 e-h	56,667 d-g	22.11 a-d
KKU99-06	57.50 fgh	69,167 ab	19.38 gh
KKU99-02	54.25 f-i	50,833 f-i	22.18 a-d
UT13	52.17 ghi	45,833 ij	22.69 abc
UT12	48.75 hi	51,667 f-i	21.33 def
KK06-501	41.50 i	43,333 j	21.70 cde
Means	64.25	56,667	21.14
F-test	**	**	**

\*\* = Significant at 0.01 probability levels. Different letters in each column show significant difference at  $P = 0.05$  by Duncan's New Multiple Range Test (DMRT).

**Table 2.** Stalk diameter, stalk length, and single stalk weight of 19 sugarcane clones at harvest.

Clones	Stalk diameter (cm)	Stalk length (cm)	Single stalk weight (kg)
MPT02-458	3.03 bcd	254.17 abc	1.7 b-f
KK07-037	2.75 g	254.08 abc	1.5 efg
KK3	3.03 abc	250.75 abc	1.8 b-e
CSB07-79	2.85 efg	256.33 ab	1.9 abc
K88-92	2.94 cde	246.83 a-d	1.4 fgh
KKU99-01	3.05 ab	216.17 def	1.6 c-g
TBy28-0941	2.89 def	251.75 abc	1.8 a-d
CSB07-219	2.92 cde	255.58 ab	1.7 b-f
MPT05-187	3.03 abc	270.92 a	2.1 a
TBy28-1211	3.10 a	189.83 f	1.6 d-g
Q229	2.49 h	227.83 b-e	1.1 h
Kps01-12	3.08 ab	237.42 b-e	1.8 a-e
TP06-419	2.90 def	244.42 a-d	1.5 efg
KKU99-03	2.78 g	209.33 ef	1.4 gh
KKU99-06	2.89 def	224.08 cde	1.5 efg
KKU99-02	3.11 a	234.75 b-e	2.0 ab
UT13	2.77 fg	235.58 b-e	1.6 c-g
UT12	2.78 fg	190.50 f	1.4 fgh
KK06-501	2.80 fg	213.08 ef	1.5 efg
Means	2.90	234.92	1.63
F-test	**	**	**

\*\* = Significant at 0.01 probability levels, Different letters in each column show significant difference at  $P = 0.05$  by Duncan's New Multiple Range Test (DMRT).

### Responses for physiological traits

The responses of 19 sugarcane clones for physiological traits were evaluated for SCMR, chlorophyll fluorescence (Fv/Fm), RWC, and stomatal conductance in the drought stress period (90 DAP) and recovery period (180 DAP). Sugarcane clones were significantly different ( $P \leq 0.01$  or  $P \leq 0.05$ ) for SCMR, RWC, chlorophyll fluorescence, and stomatal conductance in the drought stress period (90 DAP) and recovery period (180 DAP) (Table 5). The average

SCMR values were 39.11 in the drought stress period (90 DAP), and 43.97 in the recovery period (180 DAP). The average Fv/Fm values were 0.78 in the drought stress period (90 DAP), and 0.81 at the recovery period (180 DAP). The average RWC values were 83.73% in the drought stress period (90 DAP), and 97.74% in the recovery period (180 DAP). Stomatal conductance values were 147.68 m mol m<sup>2</sup>s<sup>-1</sup> in the drought stress period (90 DAP), and 392.22 m mol m<sup>2</sup>s<sup>-1</sup> in the recovery period (180 DAP).

**Table 3.** Stalk height and increased stalk height rate of 19 sugarcane clones in the drought stress period (90 DAP), recovery period (180 DAP), and full growth period (270 DAP).

Clones	Stalk height (cm)			Stalk height increased rate (cm day <sup>-1</sup> )		
	90 DAP	180 DAP	270 DAP	0-90 DAP	90-180 DAP	180-270 DAP
MPT02-458	23.00 e-h	133.25 ab	200.49 a-d	0.23 c-f	2.36 ab	0.91 abc
KK07-037	22.67 fgh	102.92 efg	177.93 b-f	0.22 def	1.88 abc	1.02 ab
KK3	20.00 h	127.08 bcd	193.05 b-f	0.20 f	1.93 abc	0.87 bcd
CSB07-79	31.50 abc	147.92 a	223.32 a	0.31 abc	1.84 bc	0.99 ab
K88-92	19.50 h	106.67 efg	189.79 b-e	0.19 f	1.93 abc	1.10 ab
KKU99-01	23.67 d-h	112.42 d-g	179.18 c-g	0.23 b-f	1.88 abc	0.86 bcd
TBy28-0941	30.75 a-d	126.33 bcd	195.17 b-f	0.30 a-d	1.51 cde	0.90 abc
CSB07-219	28.83 b-f	118.58 b-e	192.99 b-f	0.29 b-e	1.48 cde	0.98 ab
MPT05-187	32.00 ab	119.42 b-e	198.59 abc	0.32 ab	1.36 cde	1.05 ab
TBy28-1211	30.25 a-e	96.25 fgh	147.43 h	0.30 a-d	0.65 f	0.68 cd
Q229	24.92 b-h	132.67 abc	208.23 ab	0.25 b-f	2.49 a	0.97 ab
Kps01-12	27.58 b-g	126.67 bcd	190.88 b-f	0.27 b-f	1.61 cde	0.85 bcd
TP06-419	20.75 gh	113.50 c-f	192.77 a-d	0.21 ef	1.63 cd	1.08 ab
KKU99-03	27.67 b-g	77.75 h	160.11 fgh	0.27 b-f	0.99 ef	1.10 ab
KKU99-06	24.25 c-h	93.17 gh	164.81 e-h	0.24 b-f	1.74 bcd	0.96 ab
KKU99-02	31.25 abc	102.00 efg	191.15 b-e	0.31 a-d	1.19 def	1.16 a
UT13	25.08 b-h	108.42 d-g	172.34 d-g	0.25 b-f	1.67 cd	0.88 bc
UT12	21.67 fgh	115.83 b-e	160.72 gh	0.21 ef	1.94 abc	0.61 d
KK06-501	37.58 a	110.67 d-g	179.63 b-f	0.37 a	1.19 def	0.95 ab
Means	26.50	114.30	185.20	0.30	1.60	0.90
F-test	**	**	**	**	**	*

\*\* , \* = Significant at 0.01 and 0.05 probability levels, respectively, Different letters in each column show significant difference at  $P = 0.05$  by Duncan's New Multiple Range Test (DMRT).



**Table 4.** Number of tillers per stool and number of stalks per stool of 19 sugarcane clones in the drought stress period (90 DAP), recovery period (180 DAP), and full growth period (270 DAP).

Clones	Number of tillers stool <sup>-1</sup> (90 DAP)	Number of stalks stool <sup>-1</sup>		
		180 DAP	270 DAP	365 DAP
MPT02-458	3.1 de	3.3 e	4.7 cd	6.3 bc
KK07-037	3.3 cde	3.3 e	5.9 ab	7.2 a
KK3	5.6 a	4.8 ab	4.9 bcd	6.8 ab
CSB07-79	4.5 a-d	4.6 a	5.1 bcd	5.6 c-f
K88-92	4.8 abc	4.1 bcd	5.9 ab	5.6 c-f
KKU99-01	4.4 a-d	3.8 cde	5.1 bcd	5.8 cde
TBy28-0941	5.5 a	3.8 bcd	5.7 ab	5.7 c-f
CSB07-219	3.0 de	3.5 cde	5.3 bcd	4.8 ghi
MPT05-187	5.0 ab	3.8 bcd	5.5 bc	5.8 cde
TBy28-1211	2.7 e	2.8 e	4.6 bcd	5.4 d-g
Q229	3.6 cde	3.3 de	6.9 a	5.9 cd
Kps01-12	5.4 a	3.8 cde	5.5 bc	6.2 bc
TP06-419	4.8 abc	4.3 abc	4.9 bcd	5.0 f-i
KKU99-03	3.5 b-e	3.4 de	4.9 bcd	5.7 c-f
KKU99-06	4.5 abc	3.3 de	4.8 bcd	6.9 ab
KKU99-02	4.2 abc	3.3 de	5.2 bcd	5.1 e-h
UT13	4.0 b-e	3.2 de	5.1 bcd	4.6 hi
UT12	3.4 cde	3.6 cde	4.1 d	5.2 d-h
KK06-501	3.4 cde	3.3 e	4.5 cd	4.3 i
Means	4.1	3.6	5.2	5.7
F-test	**	**	*	**

\*\*, \* = Significant at 0.01 and 0.05 probability levels, respectively, Different letters in each column show significant difference at  $P = 0.05$  by Duncan's New Multiple Range Test (DMRT).

Means for SCMR, chlorophyll fluorescence, RWC, and stomatal conductance in the drought stress period (90 DAP) were lower than in the recovery period (180 DAP). Within drought stress period (90 DAP), the more interesting clones with good physiological traits were identified. MPT02-458 and CSB07-79 demonstrated high RWC and stomatal conductance, whereas KK07-037, K88-92, and KK3 had high SCMR. KKU99-01 and TBy28-0941 had high Fv/Fm and RWC.

Within the recovery period (180 DAP), the more interesting sugarcane clones were also identified. MPT02-458 had high Fv/Fm and RWC; KK07-037 had high RWC and stomatal conductance; KK3 had high Fv/Fm and stomatal conductance; and CSB07-79

had high RWC. K88-92 and TBy28-0941 had high Fv/Fm, RWC, and stomatal conductance.

### Relationships among cane yield, yield components, and physiological traits

Cane yield was significantly correlated with stalk length ( $P \leq 0.01$ ,  $r = 0.58$ ), number of stalks/stool ( $P \leq 0.01$ ,  $r = 0.62$ ), and millable cane ( $P \leq 0.01$ ,  $r = 0.63$ ); negatively correlated with total soluble solids ( $P \leq 0.05$ ,  $r = -0.39$ ); yet the correlations between cane yield and stalk diameter and single stalk weight were not significant (Figure 3). MPT02-458, KK07-037, and KK3 produced high cane yields, stalk lengths, number of stalks/stool, and millable cane; whereas KK06-501,

**Table 5.** SCMR, chlorophyll fluorescence (Fv/Fm), RWC (%), and stomatal conductance ( $\text{m mol m}^{-2}\text{s}^{-1}$ ) of 19 sugarcane clones in the drought stress period (90 DAP) and recovery period (180 DAP).

Clones	SCMR		Fv/Fm		RWC		Stomatal conductance	
	90 DAP	180 DAP	90 DAP	180 DAP	90 DAP	180 DAP	90 DAP	180 DAP
MPT02-458	39.36 bc	41.50 i	0.780 ab	0.810 bcd	85.25 a-d	97.79 abc	200.33 a-d	305.67 ef
KK07-037	39.53 abc	43.19 e-h	0.774 bc	0.807 b-e	82.99 b-f	98.23 abc	138.27 d-g	520.83 a
KK3	39.69 abc	41.78 hi	0.780 ab	0.799 def	79.65 def	97.18 c-f	157.95 c-f	460.00 a-d
CSB07-79	38.49 bcd	45.39 bc	0.774 bc	0.792 ef	85.61 a-d	98.41 ab	171.00 b-e	360.33 c-f
K88-92	41.67 ab	43.89 c-f	0.776 abc	0.803 b-f	82.44 b-f	98.49 a	113.67 efg	502.50 ab
KKU99-01	39.02 bc	44.76 b-e	0.770 bc	0.814 abc	91.33 a	97.39 b-e	132.08 d-g	347.67 def
TBy28-0941	38.21 bcd	42.47 f-i	0.783 ab	0.816 ab	85.72 a-d	98.07 abc	80.60 g	412.83 a-e
CSB07-219	37.11 cd	42.24 ghi	0.780 ab	0.812 a-d	80.59 c-f	96.53 ef	259.92 a	512.50 ab
MPT05-187	40.59 abc	44.84 bcd	0.793 a	0.805 b-f	86.22 a-d	98.10 abc	88.48 fg	500.83 abc
TBy28-1211	38.16 bcd	47.81 a	0.767 bc	0.815 ab	83.94 b-f	98.19 abc	176.83 b-e	313.17 ef
Q229	37.48 bcd	43.64 d-g	0.776 abc	0.795 ef	77.29 f	98.42 ab	118.17 efg	320.50 def
Kps01-12	43.70 a	43.93 c-f	0.771 bc	0.810 bcd	86.03 a-d	96.69 def	113.42 efg	460.17 a-d
TP06-419	34.32 d	42.83 f-i	0.773 bc	0.805 b-f	80.15 def	98.69 a	118.77 efg	377.33 b-e
KKU99-03	38.72 bc	43.61 d-g	0.785 ab	0.801 b-f	81.76 b-f	97.38 b-e	92.77 fg	296.33 ef
KKU99-06	41.09 abc	43.86 c-g	0.785 ab	0.792 f	84.49 b-e	96.23 f	175.00 b-e	311.50 ef
KKU99-02	41.02 abc	45.95 b	0.761 cd	0.800 c-f	84.50 b-e	97.82 abc	95.62 fg	530.83 a
UT13	36.95 cd	43.74 d-g	0.774 bc	0.825 a	87.35 ab	98.30 ab	103.17 efg	340.17 def
UT12	39.52 abc	44.52 b-e	0.786 ab	0.799 def	87.15 abc	97.39 b-e	227.17 abc	232.00 f
TP06-501	38.54 bcd	45.42 bc	0.743 d	0.803 b-f	78.44 ef	97.67 a-d	242.67 ab	347.00 def
Means	39.11	43.97	0.78	0.81	83.73	97.74	147.68	392.22
F-test	*	**	**	**	*	**	**	**

\*\* , \* = Significant at 0.01 and 0.05 probability levels, respectively, Different letters in each column show significant difference at  $P = 0.05$  by Duncan's New Multiple Range Test (DMRT).

UT12, and UT13 had low cane yield, stalk lengths, number of stalks/stool, and millable cane. The associations of stalk length, number of stalks/stool, and millable cane with cane yield were strong; indicating their importance as main component characters contributing to cane yield.

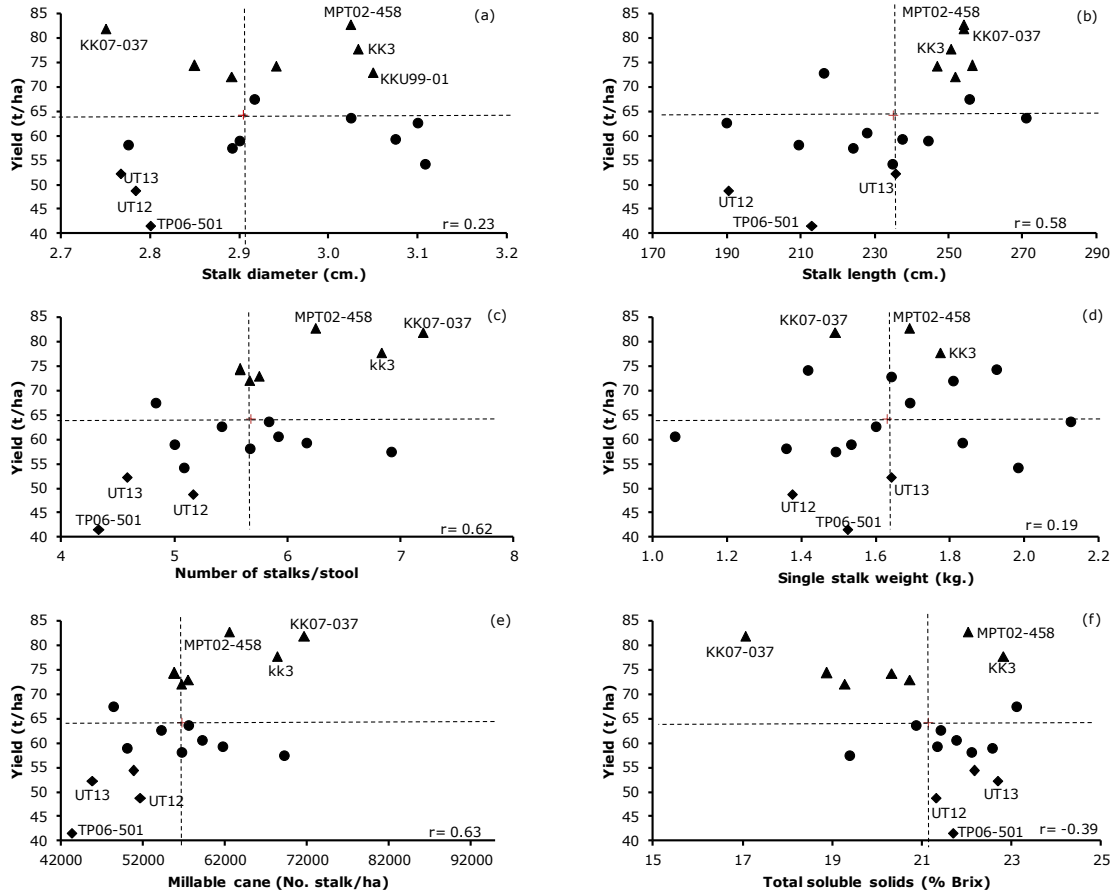
The correlation coefficients between cane yield and the physiological traits, including SCMR, Fv/Fm, RWC, and stomatal conductance, were not significant in the drought stress period (90 DAP) and the recovery period (180 DAP) (Figures 4, 5, 6 and 7). MPT02-458, KK07-037, and KK3 had high cane yields (70-85 t/ha), but low SCMR, Fv/Fm, RWC, and stomatal conductance at 90 DAP (drought stress period). All sugarcane clones demonstrated increases in SCMR,

Fv/Fm, RWC, and stomatal conductance in the recovery period (180 DAP). KK06-501, UT12, and UT13, however, produced low cane yields (40-55 t/ha); as well as low SCMR, Fv/Fm, RWC, and stomatal conductance in both the drought stress period (90 DAP) and recovery period (180 DAP).

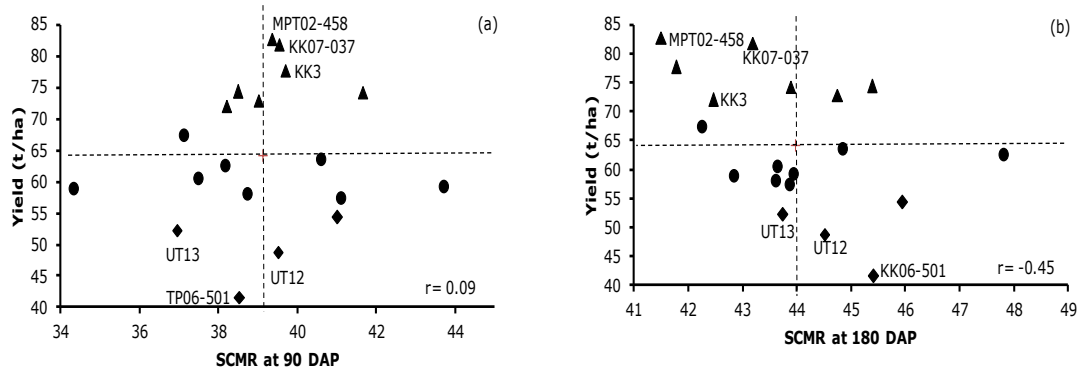
## DISCUSSION

### Cane yield and yield components

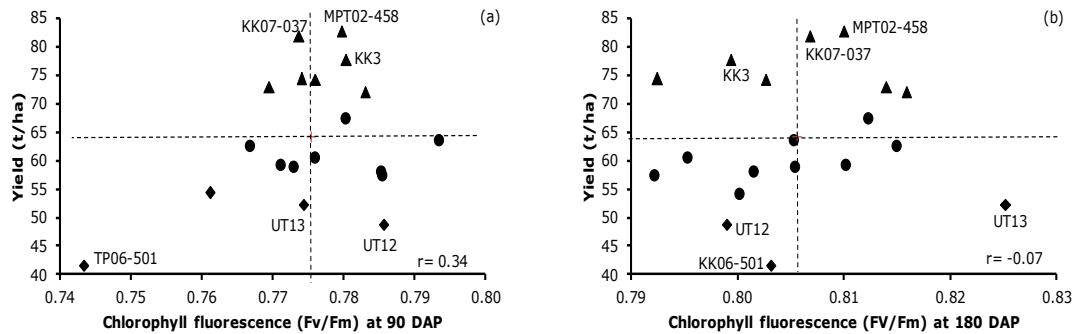
Variations in 19 sugarcane clones were evaluated for stalk diameter, stalk length, stalk height, increased stalk height rate, number of stalks/stool, number of tillers/stool, single stalk weight, millable cane, yield, and total soluble solids; which



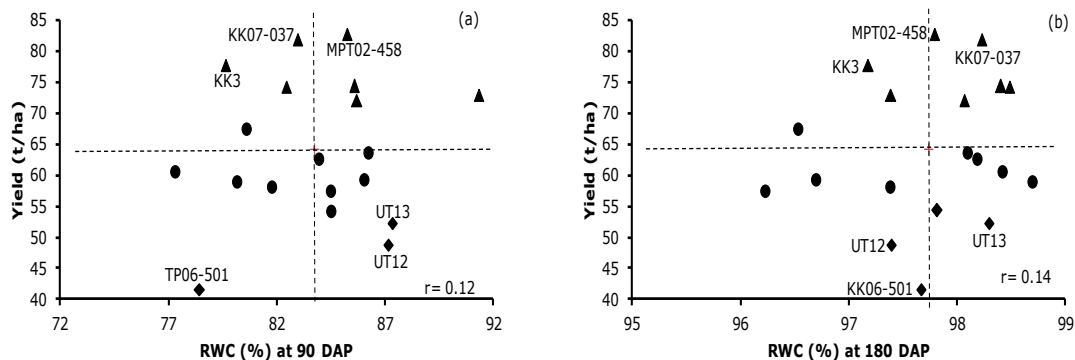
**Figure 3.** Relationships between cane yield and stalk diameter (a), stalk length (b), number of stalks/stool (c), single stalk weight (d), millable cane (e) and total soluble solids (f) in 19 sugarcane clones. (▲) indicates the clones (MPT02-458, KK07-037, KK3, CSB07-79, K88-92, KKU99-01, and TBy28-0941) with a high cane yields, (◆) indicates the clones (KKU99-02, UT13, UT12, and KK06-501) with low cane yields.



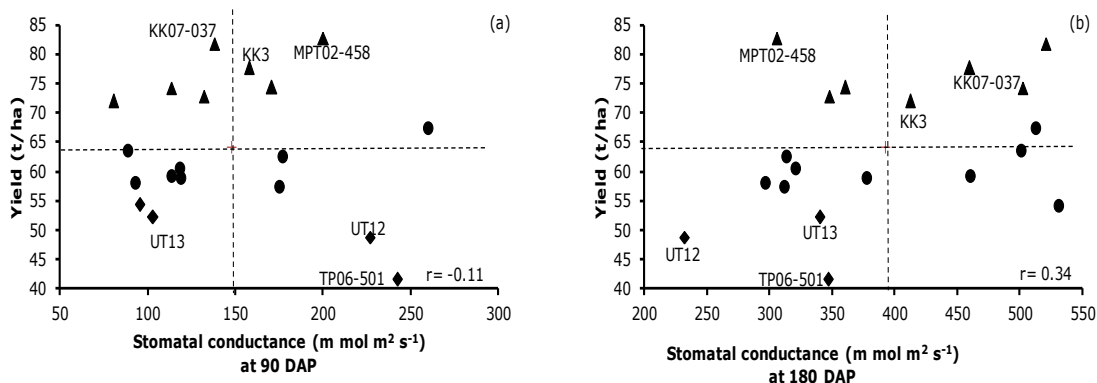
**Figure 4.** Relationships between cane yield and SCMR in 19 sugarcane clones in the drought stress period (90 DAP) (a) and recovery period (180 DAP) (b). (▲) indicates the clones (MPT02-458, KK07-037, KK3, CSB07-79, K88-92, KKU99-01, and TBy28-0941) with high cane yields, and (◆) indicates the clones (KKU99-02, UT13, UT12, and KK06-501) with low cane yields.



**Figure 5.** Relationships between cane yield and chlorophyll fluorescence in 19 sugarcane clones in the drought stress period (90 DAP) (a) and recovery period (180 DAP) (b). ( $\blacktriangle$ ) indicates the clones (MPT02-458, KK07-037, KK3, CSB07-79, K88-92, K KU99-01, and TBy28-0941) with high cane yields and ( $\blacklozenge$ ) indicates the clones (K KU99-02, UT13, UT12, and KK06-501) with low cane yields.



**Figure 6.** Relationships between cane yield and RWC in 19 sugarcane clones in the drought stress period (90 DAP) (a) and recovery period (180 DAP) (b). ( $\blacktriangle$ ) indicates the clones (MPT02-458, KK07-037, KK3, CSB07-79, K88-92, K KU99-01, and TBy28-0941) with high cane yields, and ( $\blacklozenge$ ) indicates the clones (K KU99-02, UT13, UT12, and KK06-501) with low cane yields.



**Figure 7.** Relationships between cane yield and stomatal conductance in 19 sugarcane clones in the drought stress period (90 DAP) (a) and recovery period (180 DAP) (b). ( $\blacktriangle$ ) indicates the clones (MPT02-458, KK07-037, KK3, CSB07-79, K88-92, K KU99-01, and TBy28-0941) with high cane yields, and ( $\blacklozenge$ ) indicates the clones (K KU99-02, UT13, UT12, and KK06-501) with low cane yields.

indicated the possibility of superior genotype selection within these sugarcane clones.

The drought tolerant clones in this study had differential responses for agronomic traits; i.e., stalk diameter, stalk length, number of stalks/stool, single stalk weight, millable cane, cane yield, and total soluble solids under drought stress. In this study, KK3 produced the highest stalk diameter, stalk length, number of stalks/stool, number of tillers/stool, millable cane, cane yield, and total soluble solids, but had a low rate of increased plant height.

According to Silva *et al.* (2008); sugarcane genotypes differed in stalk diameter, stalk height, stalk number, tiller number, and cane yield; while demonstrating sensitivity to drought stress. It seems likely that the tolerant genotypes might employ different mechanisms to acclimate to dehydration within the early growth stage.

The KK3 was reported to be a drought tolerant genotype under drought conditions (Khonghintaisong *et al.*, 2018). This genotype invested more assimilates on root growth in response to drought. Although KK3 lengthened its roots in order to take up more water, it seems likely that the amount of water supply to the shoot was insufficient. According to Jangpromma *et al.* (2012); KK3 performed well for rooting traits upon early drought stress, as well as in the re-watering periods. However, De-Silva and De-Costa (2004); reported that these parameters were very important for determining yield under water deficit conditions, in which the sugarcane genotypes responded differently for each trait. Thus, selection for these characteristics might be useful for sugarcane

breeding, as they are easily measured, and may allow for the selection of a large number of drought tolerant genotypes within a short period of time.

### **Responses for physiological traits**

Responses in 19 sugarcane clones were evaluated for SCMR, chlorophyll fluorescence, RWC, and stomatal conductance in both the drought stress period (90 DAP) and recovery period (180 DAP); in which the results were significantly different. Notably, SCMR, chlorophyll fluorescence, RWC, and stomatal conductance evaluated in the recovery period were higher than those evaluated in the drought stress period.

In this study, as well as in the sugarcane study performed by Jangpromma *et al.* (2010) also reported that SCMR was reduced in drought conditions at 90 DAP. Silva *et al.* (2011) found that SCMR under water deficit conditions was significantly lower than that under well-watered conditions.

Within the drought stress period (90 DAP), all sugarcane clones maintained chlorophyll fluorescence values. However, under the recovery period at 180 DAP, chlorophyll fluorescence increased. Maxwell and Johnson, 2000; found that the reduction in chlorophyll fluorescence was associated with photo-inhibition by an over reduction of PSII. Also, it is well known that a sustained decrease in chlorophyll fluorescence reflects photo-inhibitory damage in response to environmental stress.

In the current study, water deficit stress resulted in significant reductions in RWC in all sugarcane genotypes. Maintaining a relatively high RWC during mild drought is

indicative of drought tolerance (Silva *et al.*, 2014). According to Gorai *et al.* (2010); the precise knowledge of the leaf tissue water status through RWC is important for the quantification of the plant's water content. This parameter has become a key indicator for screening crops for drought-tolerant genotypes.

The low reduction in relative water content found in sugarcane may be an indication that the species is more tolerant to drought. In this study, drought at 90 DAP also reduced stomatal conductance, indicating that stomatal conductance of sugarcane is controlled by its roots; and may also involve a chemical signal (Smith *et al.*, 1999). Reduction in stomatal conductance was addressed as an effect from water deficiency (Zhao *et al.*, 2010). However, selection of plants for drought tolerance is difficult, due to the genetic complexity of drought tolerance traits (Silva *et al.*, 2011).

### **Associations of yield components and physiological traits with cane yield**

Correlations among characteristics are important for simultaneous selection of multiple traits, in which the selection of a single trait may affect others. In this study, cane yield was significantly associated with yield components; such as stalk length, number of stalks/stool, and millable cane. The relationships between cane yield and physiological traits; such as SCMR, chlorophyll fluorescence, RWC, and stomatal conductance were not significant in the drought stress period (90 DAP) as well as in the recovery period (180 DAP). The results of this study also indicated that cane yield, yield components, and physiological

traits were not interrelated. The lack of association among these traits would be possibly due to the differences in mechanisms used by each sugarcane variety to maintain high yield under drought stress.

The negative correlation of total soluble solids with cane yield is one of the major constraints in the improvement of sugarcane production (Khan, 2012). The results also demonstrated that sugarcane productivity is strongly associated with the ability to maintain higher levels of physiological functions as indicated by higher values of Fv/Fm, SPAD index, and RWC; as well as lower stomatal conductance under drought stress periods to maintain water status input by reduce water loss. Silva *et al.*, 2014; reported that the ability of sugarcane genotypes to maintain high leaf water status, and thus preserve the photosynthetic apparatus under water deficits, resulted in higher productivity values.

Moreover, sugarcane genotypes produced differential responses for morphology and physiology to variations throughout drought duration and intensity, occurring at different growth stages (Bartels and Sunkar, 2005; Smit and Singels, 2006; Da-Graca *et al.*, 2010; Inman-Bamber *et al.*, 2012; Ferreira *et al.*, 2017). Additionally, these physiological traits have a great potential as selection tools in breeding programs aimed at improving crop productivity in drought prone environments. Therefore, it is important to select physiological markers that could either confer adaptation and higher yield under water stress conditions, or produce an association with drought tolerance. These traits may potentially be used to routinely screen genotypes and

parental plants for the selection of new drought tolerant genotypes in breeding programs (Silva et al., 2011).

## ACKNOWLEDGEMENT

The research project was funded by the Northeast Thailand Cane and Sugar Research Center (NECS), Faculty of Agriculture, Khon Kaen University, Thailand and the Thailand Research Fund (TRF), Thailand. We also wish to extend our thanks to the manuscript preparation project under Faculty of Agriculture, KKU and TRF, Thailand.

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