International Journal of Agricultural Technology 2019 Vol. 15(6):997-1010
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Selection of SSR Markers for drought resistant sugarcane in Thailand

Prasitsom, C.[1,2], Jubsab, N.[1], Klomsa-ard, P.[1], Sriroth, K.[1] and Keawsompong, S.[2,3*]

[1]Mitrphol Innovation and Research Center, Mitr Phol Group, Chaiyaphum, Thailand; [2]Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand; [3]Center for Advanced Studies in Agriculture and Food, Institute for Advanced Studies, Kasetsart University, Bangkok 10900 Thailand.

Prasitsom, C., Jubsab, N., Klomsa-ard, P., Sriroth, K. and Keawsompong, S. (2019). Selection of SSR marker for drought resistance sugarcane in Thailand. International Journal of Agricultural Technology 15(6):997-1010.

Abstract Drought is the most significant environmental stress factor affecting agriculture worldwide and improving yield under drought conditions is a primary goal of plant breeding. In Thailand, sugarcane productivity decreased by approximately 12.5 tons/hectare from 2014 to 2016 as a result of drought. Drought tolerance comprises the combination of many characteristics. Indirect screening methods based on physiological and genomic traits are used to select drought-tolerant varieties with increased growth efficiency. Physiological traits observed in this study were stomatal conductance (g_s), PSII maximum quantum yield (F_v/F_m), and leaf chlorophyll content (SPAD index). These were correlated with agronomic traits such as height, weight, and a number of stalks. Fourteen candidate SSR markers were selected as genomic data to determine the grouping relationship. Under drought conditions, our results indicated that maintaining higher photosynthetic efficiency (F_v/F_m) and chlorophyll content (SPAD index) showed the potential to achieve greater growth under water stress conditions. K93-219 had the highest values of physiological traits followed by KPS01-12, UT12, MPT10-52, and MPT03-320, respectively. A phylogenetic tree of 4 SSR markers gave an interesting pattern suggesting that K93-219 and UT12 were close neighbor groups followed by MPT10-54 and KPS01-12, respectively. Results can be used to model selective varieties of sugarcane in Thailand. This research demonstrates an alternative method for screening sugarcane varieties that can adapt and grow under water stress conditions, and offers opportunities to develop breeding approaches for crop improvement in Thailand.

Keywords: SSR marker, sugarcane, physiology, drought

Introduction

In Thailand, more than 75% of the sugarcane is produced in the north-eastern region, with slightly more than half of the production located in rain-fed areas of mainly sandy soil (Ministry of Agriculture and Cooperatives, 2013). As

* **Corresponding Author**: Keawsompong S.; **Email:** fagisuk@ku.ac.th

a result, moisture content tends to decrease quickly along with fertile qualities. In addition, El Niño and La Niña events have recently been a natural global occurrence and adversely affected rainfall in Thailand. Climate change has produced variations in temperature, rain distribution, and precipitation frequency. As a result, all crops in Thailand now face varied growing conditions. One of the most pronounced changes in climatic conditions is drought.

Drought is one of the major abiotic stresses limiting global growth and development of crops including sugarcane (da Silva *et al*., 2012). Drought alters plant-water relationships via stomatal activity, carbon fixation and other plant metabolic functions which result in changes in growth and development. Stomatal closure during water stress reduces the flux of carbon dioxide into the leaf and photosynthesis declines (Flexas *et al*., 2006 and Lakshmanan and Robinson, 2014). In addition, water stress-induced leaf senescence reduces leaf area and thus water loss, thereby contributing to the maintenance of a favorable water balance at the whole plant level. As a result, photosynthesis of the whole canopy is lowered with reduced stomatal conductance (g_s) and green leaf area. From a crop production perspective, these responses negatively impact yield (Rivero *et al*., 2009). Physiological traits are directly or indirectly associated with crop growth and yields (Silva *et al*., 2007; Inman-bamber *et al*., 2012, and Wilkinson *et al*., 2012). Physiological adaptations to water stress at the whole plant level are highly complex due to the diverse responses elicited by distinct plant species to different levels of water stress and involve both deleterious and adaptive changes (Kramer and Boyer, 1995). The net effect of water deficit is largely determined by the dynamics, duration, and intensity of soil water depletion, atmospheric water demand and other prevailing environmental stresses including the stage of plant growth and phenology (Grant, 2012).

Drought mostly affects sugarcane during the summer season when the plant is at the tillering and elongation stage at around the 3rd to the 7th month after propagation. In response to drought, sugarcane displays pronounced morphological and growth responses that include a reduction in stalk and leaf elongation and accelerated leaf senescence. Together, these responses result in a decrease in biomass production and sucrose yield due to reduced rates of photosynthesis and crop growth as well as altered partitioning of assimilates between roots, leaves, structural stem material and stored sucrose (Singels *et al*., 2000; Singels and Inman-bamber, 2002; Inman-bamber, 2004, and Singels *et al*., 2010). Lost production yield associated with drought is estimated at 12.5 tons/hectare in the Thai sugarcane industry.

In response to this challenge, sugarcane breeders focus on conventional breeding of new varieties but the characteristics of drought tolerance are

complicated. Moreover, conventional breeding takes a long time with high investment to screen and select an elite variety. Sugarcane is an annual crop and 7-10 years are required to propagate a new variety (Juttupoornpong *et al*., 2012). Indirect methods for the selection of new sugarcane cultivars offer the potential to select and screen elite varieties based on the use of phenotypic data related to drought characteristic traits.

The candidate varieties were planted in a greenhouse for a period of 8 months. SSR markers were selected using a phylogenic tree that related physiological data as stomatal conductance (g_s), PSII maximum quantum yield (F_v/F_m), leaf chlorophyll content (SPAD index), and agronomic traits such as height, number of stalks per pot, and weight.

The objective was to select SSR markers for drought-resistant sugarcane in Thailand. Conventional methods use sexual reproduction to breed many seedlings. Sugarcane was planted as single seeds in a row and the seedlings were transplanted into individual pots. Normally, potential sugarcane was selected by physiological data talking for several years. SSR marker can be indicated trait associate to reduce the time for selection characteristic of sugarcane at least 30 percent on a breeding program and remained varieties to have the constant characteristic.

Materials and Methods

Plant material and growth conditions

Sugarcane (*Saccharum officinarum* L. varieties K93-219, KPS01-12, KK3, PHIL66-07, MPT10-54, UT12, MPT03-320, SP50, TBY20-2248, and KPK98-40) were planted in a greenhouse at Mitr Phol Sugarcane Research Center Phu Khiao district, Chaiyaphum Province, Thailand. Each variety was planted by RCDB (Randomized complete block design) in three pots of 15-inch diameter and grown under two conditions drought (D) and irrigated (I). Fertilizer was applied three times, 1) on the planting day at ratio 312.5 kg of 16-16-8 NPK per hectare, 2) at 3 months with ratio 312.5 kg of 21-7-18 NPK per hectare, and 3) at 4.5 months with ratio 156.25 kg of 21-7-18 NPK per hectare.

Measurement of physiological and morphological parameters

Measurements of phenotypic data collected at the 3rd, 5th and 7th month stages of sugarcane growth were edited from Somkit *et al*. (2007); Sharma (2009), and da Silva *et al*. (2012). Each pot in the greenhouse was used to collect the data in each block. The germination rate of sugarcane was measured

at 45 days. Number of stalks per pot, the diameter of each stalk, number of internodes, and height of sugarcane were measured at the 3rd, 5th and 7th month stages of growth.

PSII maximum quantum yield (F_v/F_m): Chlorophyll fluorescence was measured by Handy PEA (Hansatech Instruments, UK) using at least four leaves per sample. The leaves were shielded from light for 15-30 min using leaf clips before measurement following the method adjusted from Kautsky and Hirsch (1931) for use in sugarcane.

Stomatal conductance (g_s): Measurements were made using three Porometer AP-0 Instruments (Delta-T Devices, Cambridge, UK) and average values were calculated. Usability concerned daily temperature and humidity, with appropriate storage time between 8 and 14 hours. The method was adjusted from Stiles (1970) for use in sugarcane.

Leaf chlorophyll content (*SPAD index*): SPAD index was measured using SPAD-502 plus (Konica Minolta, Japan). Average values were measured in the middle portion of the four youngest fully expanded leaves (Dray *et al*., 2012).

Molecular analysis

Fourteen SSR genetic markers were selected by a literature review that related to drought in sugarcane and 10 varieties in the greenhouse were extracted for DNA. PCR reaction was improved from the protocol of Hoisington (1992). Twenty microliters consisted of 5 µl of 10 ng/µl DNA sample, 1.0 µl of 25 mM $MgCl_2$, 1.2 µl of 2.5 mM dNTP Mix, 2.0 µl of 10X *Tag* buffer, 5.0 µl of 1.0 µM for forward and reverse primers, 2.0 µl of %100 glycerol, 0.2 µl of 5 U/µl *Tag* polymerase, and 3.6 µl of ddH_2O. PCR amplification was performed by T100 Thermal Cycle Bio-Rad (Bio-Rad, CA, USA) in a program of 94°C 3 min, 35 cycles of 94°C 1 min, annealing 2 min, 72°C 1 min, extension at 72°C 5 min and kept at 4°C. Annealing temperatures ranged between 50 and 56°C depending on each SSR primer. A pre-test was performed by agarose gel electrophoresis and then the PCR product was analyzed by the ZAG system (ZAG DNA Analyzer AATI, CA, USA).

Statistical analysis

The greenhouse experiment was arranged in a randomized complete block design with three replications. Plants were grown under well-watered and water stress conditions. All phenotypic data were clarified and analyzed by SPSS (IBM, NY, USA) using the general linear model (GLM), ANOVA and

Tukey's test at a 95% confidence interval. Then, the average of the three values was reported as the measured value with the standard deviation. All genetic data from ZAG were analyzed by ProSize software (AATI, CA, USA). Analyzed data were exported to Microsoft Excel® and GeneTools (Syngene, MD, USA) and then a phylogenetic tree was constructed by GeneDirectory (Syngene, MD, USA). The phylogenetic tree was compared with phenotypic data to select SSR marker groups related to drought characteristics. Highest score groups from the phenotypic data were likely to give a high yield of sugarcane in drought conditions, while lowest score groups were likely to give a low yield of sugarcane in drought conditions. SSR markers were combined and assessed to form a phylogenetic tree that gave the best pattern to explain both agronomic and physiological results.

Results

Growth and physiology in drought conditions

Sugarcane was planted in a greenhouse using three replications of (RCBD), and data were collected during the 3rd, 5th and 7th month of sugarcane growth. All data were analyzed by SPSS using the general linear model, ANOVA, and Tukey's test at a 95% confidence interval. Significant effects ($P<0.05$) were observed for different interaction on six physiological parameters as a number of stalks per plant, height, weight, stomatal conductance, photosynthesis efficiency and chlorophyll content (Table 1). A few sugarcane stalks showed significance at the 3rd and the 5th months but no significance at the 7th month. Varieties having the greatest characteristics of the stalk were KK3 at the 3rd month and MPT10-54 at the 5th month. The one-way interaction of variance components on height, photosynthesis efficient, and chlorophyll content were significant at the 3rd, 5th and 7th months of growth. PHIL66-07 was the main character in the height parameter for all months; however, some varieties recorded a high score in some months e.g. K93-219 and MPT10-54 in the 5th month, and KPS01-12 and MPT10-54 in the 7th month. For photosynthesis efficiency, K93-219, MPT03-320, SP50, and TBY20-2248 had the highest score in the 5th month, while MPT10-54 gave the highest score in the 7th month. UT12 had the highest score of chlorophyll content in the 7th month. The treatment characteristic was not significant for weight and stomatal conductance. The highest average score of stomatal conductance was recorded in KK3 and MPT10-54 in the 5th and 7th month, respectively. On the other hand, the highest average score of weight was recorded in SP50.

Genetic markers of drought tolerance with sugarcane

Fourteen SSR markers were tested for sugarcane genetic diversity and separated into five groups as ABA signaling pathway, protein signaling, electron transport hemoprotein, photosynthesis, and hydrogen peroxide removal (Table 2). The dendrogram of the SSR based analyses of 10 sugarcane cultivars that exhibited high diversity of DNA polymorphism clearly discriminated between genotypes (Figure 1). SSR markers amplify specific regions with different patterns between the groups. The dendrogram was constructed using four markers (SSR9, SSR80, A19, A70) that related to drought function that ABA signaling pathway and hydrogen peroxide removal. Four groups of genetic patterns were separated in the dendrogram. KK3 showed the most difference at 0.66 coefficient in the first group. The second group consisted of PHIL66-07 and MPT03-320 at 0.716 coefficient. TBY20-2248 and KPK98-40 showed similarities at 0.79 coefficient in the third group together with the SP50 cultivar. The last group consisted of K93-219, UT12, MPT10-54, and KPS01-12.

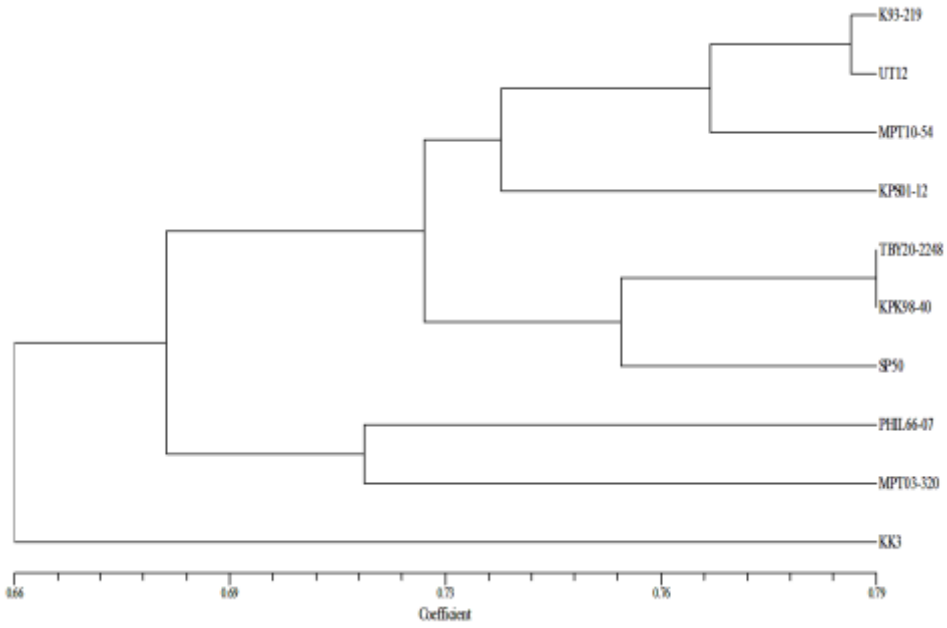

Figure 1. UPGMA dendrogram derived using Jaccard coefficient of similarity based on four SSR genetic distance data of 10 sugarcane varieties

Table 1. Sugarcane phenotypes in the greenhouse

Treatment	Stalks per plant			Height			Weight	Stomatal conductance		Photosynthesis efficiency		Chlorophyll content
	3M	5M	7M	3M	5M	7M	7M	5M	7M	5M	7M	7M
K93-219-D	7±0.71$^{ABC D}$	5±1.66^{ABC}	4±0.97^{A}	41.78±6.04^{BC}	89.89±12.71^{A}	134.78±12.96^{ABCD}	4.33±1.31^{A}	2.48±2.20^{A}	3.01±2.24^{A}	649.1±19.69^{AB}	774.6±11.08^{ABC}	35.51±6.55^{ABC}
K93-219-I	6±1.17$^{ABC DE}$	5±1.50^{ABC}	4±1.58^{A}	33.00±4.66$^{CD EF}$	76.94±11.96^{ABC}	146.44±19.87^{ABC}	3.78±0.68^{A}	3.95±2.74^{A}	3.54±2.67^{A}	742.3±3.11^{A}	789.2±7.08^{AB}	31.23±5.14^{BC}
KK3-D	9±4.00^{A}	5±2.51^{ABC}	4±1.50^{A}	35.56±5.77$^{CD E}$	81.33±8.43$^{A B}$	105.67±12.31^{CDEF}	3.67±0.44^{A}	5.96±4.51^{A}	2.11±1.24^{A}	542.4±18.93^{B}	777.2±13.22^{ABC}	34.19±2.71^{BC}
KK3-I	5±2.06^{DE}	5±1.87^{ABC}	4±1.48^{A}	28.22±5.52$^{DE F}$	63.78±10.27^{ABC}	141.78±13.65^{ABC}	4.22±1.13^{A}	3.85±3.27^{A}	2.28±1.32^{A}	706.2±10.56^{AB}	792.7±11.28^{AB}	35.44±6.71^{ABC}
KPK98-40-D	5±1.33^{CDE}	4±1.73^{ABC}	3±1.09^{A}	39.11±7.85$^{AB CD}$	67.44±25.74^{ABC}	92.44±22.90^{DEF}	3.28±0.17^{A}	2.74±2.35^{A}	2.78±1.87^{A}	648.2±11.38^{AB}	774.9±16.91^{ABC}	33.91±4.66^{BC}
KPK98-40-I	5±1.12^{CDE}	4±1.69^{ABC}	4±0.73^{A}	32.22±7.21$^{CD EF}$	56.21±17.39^{BCD}	119.33±13.13^{ABCDEF}	3.56±0.34^{A}	4.14±4.02^{A}	3.48±2.42^{A}	698.9±7.89^{AB}	780.9±15.61^{ABC}	31.62±4.15^{BC}
KPS01-12-D	7±1.81$^{ABC DE}$	5±1.32^{ABC}	4±1.73^{A}	39.67±4.42$^{AB C}$	90.56±6.88^{A}	119.11±28.81^{ABCDEF}	4.33±0.75^{A}	4.23±2.19^{A}	1.25±0.74^{A}	623.6±12.32^{AB}	776.8±17.85^{ABC}	33.91±5.44^{BC}
KPS01-12-I	7±2.06$^{ABC DE}$	6±2.39^{ABC}	4±1.00^{A}	40.11±9.53$^{AB C}$	70.04±25.99^{ABC}	160.78±15.65^{A}	4.00±0.47^{A}	3.30±1.88^{A}	3.35±3.04^{A}	570.8±22.09^{AB}	776.7±12.87^{ABC}	33.80±2.56^{BC}
MPT03-320-D	7±1.22$^{ABC DE}$	5±1.51^{ABC}	3±0.53^{A}	35.56±3.43$^{CD E}$	69.22±9.77$^{A BC}$	91.22±20.36^{EF}	3.44±1.45^{A}	1.78±1.60^{A}	1.68±1.24^{A}	722.2±4.72^{AB}	780.2±8.67^{ABC}	33.30±2.72^{BC}
MPT03-320-I	6±1.33$^{ABC DE}$	5±2.29^{ABC}	4±0.73^{A}	30.94±4.13$^{CD EF}$	54.22±12.81^{BCD}	113.00±22.37^{BCDEF}	3.56±0.54^{A}	4.78±4.18^{A}	3.91±2.89^{A}	730.6±1.86^{A}	778.3±9.55^{ABC}	31.41±6.60^{BC}
MPT10-54-D	8±1.22^{ABC}	6±1.13^{ABC}	5±1.39^{A}	37.67±4.12$^{BC D}$	90.78±8.70^{A}	114.00±34.84^{BCDEF}	4.78±1.48^{A}	3.41±1.91^{A}	2.51±2.20^{A}	641.2±12.52^{AB}	782.1±21.85^{ABC}	33.83±4.66^{BC}
MPT10-54-I	7±2.69$^{ABC D}$	7±3.77^{A}	4±1.13^{A}	33.78±5.52$^{CD EF}$	61.72±18.67^{ABCD}	159.22±15.58^{A}	4.44±0.25^{A}	5.13±5.02^{A}	4.46±3.82^{A}	680.4±9.51^{AB}	796.9±4.46^{A}	29.60±3.73^{C}
PHIL66-07-D	5±2.05^{DE}	4±0.71^{BC}	4±2.12^{A}	46.89±9.06^{AB}	92.00±13.01^{A}	134.11±46.95^{ABCDE}	4.00±0.42^{A}	3.39±2.96^{A}	2.02±0.82^{A}	667.9±9.62^{AB}	769.9±21.99^{BC}	33.49±6.28^{BC}
PHIL66-07-I	4±1.74^{E}	5±2.65^{ABC}	4±1.32^{A}	50.22±15.47^{A}	79.92±41.33^{AB}	158.78±12.81^{A}	3.67±0.50^{A}	1.80±1.74^{A}	2.35±1.26^{A}	698.2±10.40^{AB}	791.1±8.81^{AB}	32.86±3.14^{BC}
SP50-D	9±1.83^{AB}	7±2.35^{AB}	4±0.50^{A}	40.00±9.45$^{AB C}$	81.33±17.36^{AB}	112.89±29.81^{BCDEF}	3.67±0.42^{A}	4.97±4.33^{A}	3.64±3.41^{A}	682.7±10.28^{AB}	776.7±14.55^{ABC}	34.31±3.22^{BC}
SP50-I	6±1.20$^{BCD E}$	6±1.36^{AB}	5±1.22^{A}	37.67±4.33$^{BC D}$	63.08±20.90^{ABC}	148.89±30.18^{AB}	5.33±1.49^{A}	4.14±3.37^{A}	2.09±1.58^{A}	744.3±2.76^{A}	785.6±12.44^{ABC}	36.39±3.19^{ABC}
TBY20-2248-D	6±3.12$^{BCD E}$	2±2.00^{C}	4±0.94^{A}	24.33±4.23^{F}	30.00±26.41^{D}	86.00±26.91^{F}	3.50±0.31^{A}	5.70±2.62^{A}	1.74±0.89^{A}	684.8±15.02^{AB}	779.0±15.40^{ABC}	36.07±3.89^{ABC}
TBY20-2248-I	6±2.74$^{BCD E}$	6±3.35^{AB}	4±1.50^{A}	26.33±3.67^{EF}	45.89±24.89^{CD}	93.47±51.58^{DEF}	4.42±0.48^{A}	3.50±2.43^{A}	1.58±0.78^{A}	746.8±4.91^{A}	782.2±11.61^{ABC}	34.32±5.16^{BC}
UT12-D	7±0.93$^{ABC D}$	6±1.01^{ABC}	5±1.54^{A}	33.67±5.07$^{CD EF}$	70.22±10.79^{ABC}	115.56±23.10^{BCDEF}	4.89±1.86^{A}	2.90±2.17^{A}	1.28±0.61^{A}	601.4±6.34^{AB}	766.2±14.54^{C}	38.31±3.04^{AB}
UT12-I	7±0.87$^{ABC DE}$	6±1.41^{AB}	5±1.33^{A}	34.89±5.44$^{CD EF}$	60.56±15.95^{ABCD}	135.56±26.14^{ABCD}	4.56±0.95^{A}	2.18±1.34^{A}	3.05±2.61^{A}	727.0±6.34^{AB}	779.3±5.74^{ABC}	42.20±4.57^{A}
SD	0.8876	0.9816	0.5995	3.1422	9.0312	12.174	0.4302	1.6705	1.0286	5.27	6.4398	2.1819
CV	3.1443	3.4773	2.1239	11.132	31.994	43.129	1.5239	5.9181	3.6439	18.67	22.814	7.7296
Significant	*	*	NS	*	*	*	NS	NS	NS	*	*	*

M = month, NS = not significant, D = drought condition, I = irrigated condition

Table 2. SSR markers related to drought response in sugarcane

No	Marker Name		Primer sequence (5'-3')	Range	Target	Significance	Reference	Sequence repeat*
1	SSR9	(F)	AAGAAAAGGAGGGCCAAAAA	204-320	Proteinase phosphatase 2c homolog (ABA)	Part of the ABA signaling pathway	(Sharma, 2009)	
		(R)	GCCAGGCAAGAGGATAAAAA					
2	SSR80	(F)	GTTCCCACCGCTGTCATC	229	Cysteine protease component of protease inhibitor complex (ABA)	Part of the ABA signaling pathway	(Sharma, 2009)	gtc, cgc
		(R)	TACGAGCACGTGTCCAACTC					
3	SSR230	(F)	TTGTGCTGATGTTTCCTGCT	200-380	Patatin like protein		(Sharma, 2009)	ta, at
		(R)	CAAGAGAAGATGCCATTAGCC					
4	SSR924	(F)	CCGAGTGTCCTCATCGCAGAAC	200-300	Auxin-independent growth promoter (ABA)	Part of the ABA signaling pathway	(Sharma, 2009)	cgc, cctcgc
		(R)	CTCTAGTCTCTTCATAACCTCTC					
5	SCM7	(F)	ACGGTGCTCTTCACTGCT	157-169			(Lu *et al*., 2015)	tgcg
		(R)	GGGCATACTTCCTCCTCTAC					
6	ESTB100	(F)	CCACGGGCGAGGACGAGTA	268	22 kDa drought-inducible protein	Protein signaling	(Oliveira *et al*., 2009)	ta, cgg
		(R)	GGGTCCTTCTTCGCCTCGTG					
7	ESTB130	(F)	GCCCAGGTAATTATCCAGACTC	124	Putative auxin response factor 7a		(Oliveira *et al*., 2009)	gca
		(R)	GCTGTTGCTCACTGGTTCC					
8	ESTC82	(F)	GGCGGCGGCTGGCTGGAT	149	Cytochrome b5	Electron transport hemoproteins	(Oliveira *et al*., 2009)	gcc, gca, caac
		(R)	GATTTGTGGCTGGCGGAAGTGGAC					
9	ESTC83	(F)	ATTTGTGGCTGGCGGAGGTGGAC	149	Cytochrome b5	Electron transport hemoproteins	(Oliveira *et al*., 2009)	caac, gcgt, cta
		(R)	GGCTGGCGGCTGGCTGGAT					
10	ESTC110	(F)	ACATGATCGCCGTCCTCTG	138	Putative cytochrome p450		(Oliveira *et al*., 2009)	
		(R)	GCAAAGGCAGAAAAAGGTGTT					
11	ESTC117	(F)	GGGAGCGACGAACTGACG	295	Chlorophyll a/b-binding protein	Photosynthesis	(Oliveira *et al*., 2009)	acc, ccgt
		(R)	GATCCCGTCGCCAACAAC					
12	ESTA19	(F)	CGCACCCGTTGACGAAGCAGT	191	Peroxidase 7	Hydrogen peroxide removal	(Oliveira *et al*., 2009)	ct, gc
		(R)	GTTCCTCGCGCTCCTCTGCT					
13	ESTA70	(F)	GATGGAACCTGAAGATGAAGAGCA	175	Peroxidase	Hydrogen peroxide removal	(Oliveira *et al*., 2009)	tc
		(R)	CCGGCCGGAGCACAGACG					
14	ESTB82	(F)	CGTCGATCGAGATGAAGAAGG	263	Putative peroxidase		(Oliveira *et al*., 2009)	tatg, gc, cgt, agct, ctag, acg
		(R)	GAAGCAGTCGTGGAAGTGGAG					

Discussion

Normally, sugarcane is harvested once per year, with the drought season from March to April (tillering stage) and May to August (elongation stage) (Thai Encyclopedia for Youth, 1980). Lacking water at both stages significantly impacted sugarcane yield. On the other hand, drought stress of sugarcane from November to February stimulates increased soluble sucrose to crush in the sugar factory (Juttupoornpong *et al*., 2012). Similar to the greenhouse experiment, most varieties showed that lack of water activated the pathway of the drought stress response. The main characteristics of sugarcane under drought stress comprise a complex response consisting of both agronomic and physiological traits to adapt to drought conditions (Khonghintaisong, 2017). Drought stress reduced the stalk diameter and biomass (Jangpromma *et al*., 2012) with reduced numbers of stalks (Robertson *et al*., 1999). In the absence of water, sugarcane color intensity value (SPAD) relates to the amount of chlorophyll in the leaves (Jangpromma *et al*., 2010) which promotes the creation of food through photosynthesis. Sugarcane varieties that are not resistant to water deficiency have low SPAD values. Moreover, relative water content, respiration rate and value of stomatal conductance in leaves of drought-resistant varieties have a better response to drought than weak varieties (Graca *et al*., 2010).

Three parameters (number of stalks per plant, height, and weight) were related to drought response and these were measured during the 3rd, 5th and 7th months of sugarcane grown in the greenhouse (Table 1). These agronomic traits have an important impact on the total yield of sugarcane in harvesting season (Chaves *et al*., 2002). After the elongation stage, sugarcane growth enters the sucrose accumulation stage when growth rate decreases and total yield becomes invariable. MPT10-54 showed good potential to survive in drought conditions with a higher number of stalks under drought treatment than irrigation treatment in more than 50% of all treatment varieties. Moreover, MPT10-54 in drought conditions had the second highest weight parameter after UT12. MPT10-54 in drought conditions showed decreased growth rate (height) in the 7th month, similar to all treatment varieties but higher than the irrigation condition in the 3rd and 5th months as K93-219, KK3, KPK98-40, MPT03-320, and SP50. On the other hand, K93-219 showed some interesting patterns as the high value of height in drought conditions more than in irrigated conditions in the drought period of the 3rd to the 5th months, then growth rate reduced after irrigation during the 7th month, similar to KPS01-12. Drought varieties should remain yield or increase growth rate in drought condition comparing with the normal condition, so the number of stalks per plant, height, and weight can be criteria to select drought characteristic (Ribeiro *et al*., 2013). Thus, KPS01-12, K93-

219, MPT10-54, and UT12 showed drought agronomic traits with higher yield in drought conditions than in irrigation conditions.

Another three parameters as SPAD, stomatal conductance, and photosynthesis efficiency were measured during the 5^{th} and 7^{th} months in the greenhouse. Some varieties had the potential to survive in drought conditions such as K93-219, MPT10-54, and MPT03-320 by growth rate because their value of stomatal conductance in drought conditions was less than in irrigation conditions. The stomatal conductance parameter affects stoma opening in the sugarcane leaves (Pirasteh-Anosheh *et al*., 2016). A high value of stomatal conductance parameter indicates high water loss to the environment which is the worst scenario in drought conditions (Basnayake *et al*., 2012). However, when sugarcane grows continuously it requires water for photosynthesis (Dodd, 2003). The photosynthesis efficiency parameter determines whether drought stress sugarcane can survive (Wang *et al*., 2018). KPS01-12 and MPT03-320 had high photosynthesis rates and they produced biomass as height. It has been reported that increasing chlorophyll content promotes sugarcane growth in drought conditions (Tripathi *et al*., 2019). K93-219, KPK98-40, KPS01-12, MPT03-320, MPT10-54, PHIL66-07, and TBY20-2248 had higher chlorophyll contents in drought conditions than irrigation conditions, while K93-219, MPT10-54, KPS01-12, and MPT03-320 had drought physiological response related with phenotype to give higher yield in drought conditions than in irrigation conditions.

Four SSR markers as SSR9, SSR80, A19, and A70 were analyzed by the Jaccard coefficient and UPGMA clustering method. Separate sugarcane varieties were grouped into patterns related to physiological data. Kanagaraj *et al*. (2010) reported that using more SSR marker-related trait responses improved the accuracy of predicting candidate varieties. Phylogenetic tree constructed by SSR marker analysis can relate with trait form result of cluster analysis. Each variety may have same or like the relationship between them and presence of specific effect within the group which could be due to population structure effect to closely or so far (Tabkhkar *et al*., 2018). Thus, this can support the hypothesis that the marker-trait association is independent of population structure. Physiological traits showed that each sugarcane variety had different performance in each period. KK3 as the control treatment gave a total performance in drought conditions less than MPT10-54, K93-219, MPT03-320, and KPK98-40, while K93-219 and MPT10-54 had the highest agronomical traits. Conversely, MPT03-320, TBY20-2248, and KPK98-40 had the highest physiological traits focusing on specific traits. For genetic data, results showed that K93-219 and UT12 had a close specific relationship, followed by MPT 10-54 and KPS01-12. This group showed drought efficiency

for adaptation to drought stress. The second group as KK3, MPT 03-320, and PHIL66-07 were insensitive to drought stress, while moderate drought stress was shown by the group of TBY20-2248, KPK98-40, and SP50. Four markers were related with drought function via the ABA signaling pathway and hydrogen peroxide removal. Drought is one of the most severe abiotic factors restricting plant growth and yield. Numerous gene functions in drought response are regulated by abscisic acid (ABA) dependent and independent pathways (Liu *et al*., 2018). Hydrogen peroxide (H_2O_2) functions as a signal molecule in plants under abiotic and biotic stress to improving drought tolerance (Guler and Pehlivan, 2016). Changes in H_2O_2 response to environmental conditions, in parallel with changes in abscisic acid (ABA) and oxidative stress markers, together with lignin accumulation, xylem, and sclerenchyma differentiation, and leaf area were also investigated (Jubany-Mar í *et al*., 2009). In Thailand, drought affects many regions and reduces sugarcane yield by at least 2 ton/rai. Effective sugarcane candidate varieties for drought adaptation may be able to resolve this problem and offer increased benefits to farmers. Our results suggested that four efficient SSR markers can be rearranged in Thai sugarcane varieties to relate with physiological data.

Acknowledgments

The authors are grateful to Mitr Phol Group, the Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University for providing access to laboratory equipment and instruments.

Funding: This study was supported by a Master of Science Program in Biotechnology from Kasetsart University (Study Code: 5915000754) and funded by The Thailand Research Fund (TRF)-Research and Researchers for Industries (RRI) (Grant No: MSD60I0065) awarded to Asst. Prof. Suttipun Keawsompong, Ph.D.

Compliance with Ethical Standards

Conflict of interest: All authors of this research paper declare no conflicts of interest.

References

Basnayake, J., Jackson, P. A., Inman-Bamber, N.G. and Lakshmanan, P. (2012). Sugarcane for Water-Limited Environments. Genetic Variation in Cane Yield and Sugar Content in Response to Water Stress. Experimental Botany, 63:6023-6033.

Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P. P., Osorio, M. L., Carvalho, I., Faria, T. and Pinheiro, C. (2002). How Plants Cope with Water Stress in the Field. Photosynthesis and Growth. Annals of Botany, 89:907-916.

da Silva, P. P., Soares, L., da Costa, J. G., da Silva Viana, L., de Andrade, J. C. F., Gonçalves, E. R., dos Santos, J. M., de Souza Barbosa, G. V., Nascimento, V. X.,. Todaro, A. R., Riffel, A., Grossi-de-Sa, M. F., Barbosa, M. H. P., Sant'Ana A. E. G.and Neto, C. E. R. (2012). Path Analysis for Selection of Drought Tolerant Sugarcane Genotypes through Physiological Components. Industrial Crops and Products, 37:11-19.

Dodd, I. C. (2003). Hormonal Interactions and Stomatal Responses. Plant Growth Regulation, 22:32-46.

Dray, F. A., Center, T. D. and Mattison, E. D. (2012). In situ estimates of water hyacinth leaf tissue nitrogen using a SPAD-502 chlorophyll meter. Aquatic Botany, 100: 72-75.

Flexas, J., Bota, J., Galmes, J., Medrano, H. and Ribas-carbo, M. (2006). Keeping a Positive Carbon Balance under Adverse Conditions: Responses of Photosynthesis and Respiration to Water Stress. Physiologia Plantarum, 127:343-352.

Graca, J. P., Rodrigues, F. A., Farias, J. R. B., Oliveira, M.C.N., Hoffmann-Campo, C. B. and Zingaretti, S. M. (2010). Physiological parameters in sugarcane cultivars submitted to water deficit. Brazilian Journal of Plant Physiology, 22:189-197.

Grant, O. M. (2012). Understanding and Exploiting the Impact of Drought Stress on Plant Physiology, *In* P. P. AHMAD, M. N. V., eds. Springer New York. Springer, New York.

Guler, N. S. and Pehlivan, N. (2016). "Exogenous low-dose hydrogen peroxide enhances drought tolerance of soybean (*Glycine max* L.) through inducing antioxidant system." Acta Biol Hung, 67:169-183.

Hoisington, D. (1992). Laboratory protocals. CIMMYT Appiled Molecular Genetics Laboratory. CIMMYT, Mexico.

Inman-bamber, N. G. (2004). Sugarcane Water Stress Criteria for Irrigation and Drying Off. Field Crops Research, 89:107-122.

Inman-bamber, N. G., Lakshmanan, P. and Park, S. (2012). Sugarcane for Water-Limited Environments: Theoretical Assessment of Suitable Traits. Field Crops Research, 134:95-104.

Jangpromma, N., Thammasirirak, S., Jaisil, P. and Songsri, P. (2012). Effects of drought and recovery from drought stress on above ground and root growth, and water use efficiency in sugarcane (*Saccharum officinarum* L.). Australian Journal of Crop Science, 6:1298-1304.

Jangpromma, N., Songsri, P., Thammasirirak, S. and Jaisil, P. (2010). Rapid assessment of chlorophyll content in sugarcane using a SPAD chlorophyll meter across different water stress conditions. Asian Journal of Plant Sciences, 9:368-374.

Jubany-Marí, T., Munné-Bosch, S., López-Carbonell, M. and Alegre, L. (2009). "Hydrogen peroxide is involved in the acclimation of the Mediterranean shrub, *Cistus albidus* L., to summer drought." Journal of Experimental Botany, 60:107-120.

Juttupoornpong, S., Sirisink, S., Suchato, W., Thumthong, P., Lertprasertrat, K., Duanmeesuk, U. and Sukhnimitre, M. (2012). Sugarcane Varietal Improvement of Subhan Buri Agricultural Research and Development Center. Khon Kaen Agriculture Journal, 3:1-7.

Kanagaraj, P., Prince, S., Annie Sheeba, J., Biji, K. R., Babu Paul, S., Alagarswamy, S. and Ranganathan, C. (2010). Microsatellite markers linked to drought resistance in rice (*Oryza sativa* L. Current science, 98:836-839.

Kautsky, H. and A. Hirsch (1931). Chlorophyllfluoreszenz und Kohlensäureassimilation. Naturwissenschaften, 19: 964.

Khonghintaisong, J., Songsri, P. and Jongrungklang, N. (2017). Growth and Physiological Patterns of Sugarcane Cultivars to Mimic Drought Conditions in Late Rainy Season System. Naresuan University Journal, 25:2

Kramer, P. J. and Boyer, J. S. (1995). Water Relations of Plants and Soils. Academic Press, Book Marketing Department, San Diego, US.

Lakshmanan, P. and Robinson, N. (2014). Stress Physiology: Abiotic Stresses. Sugarcane: Physiology, Biochemistry, and Functional Biology. John Wiley & Sons Ltd.

Liu, S., Lv, Z., Liu, Y., Li, L. and Zhang, L. (2018). Network analysis of ABA-dependent and ABA-independent drought responsive genes in *Arabidopsis thaliana*. Genetics and Molecular Biology, 41:624-637.

Lu, X., Zhou, H., Pan, Y. B., Chen, C. Y., Zhu, J. R., Chen, P. H., Li, Y. R., Cai, Q. and Chen, R. K. (2015). Segregation analysis of microsatellite (SSR) markers in sugarcane polyploids. Genet Mol Res, 14:18384-18395.

Ministry of Agriculture and Cooperatives. (2013). Land Use Statistics Whole Kingdom 2003-2013. Available Source: http://service.nso.go.th/nso/web/statseries/statseries14.html, October 5, 2016.

Oliveira, K. M., Pinto, L. R., Marconi, T. G., Mollinari, M., Ulian, E. C., Chabregas, S. M., Falco, M. C., Burnquist, W., Garcia, A. A. and Souza, A. P. (2009). Characterization of new polymorphic functional markers for sugarcane. Genome, 52:191-209.

Pirasteh-Anosheh, H., Saed-Moucheshi, A., Pakniyat, H. and Pessarakli, M. (2016). Stomatal responses to drought stress: 24-40. Rivero, R.M., V. Shulaev, and E. Blumwald. 2009. Cytokinin-Dependent Photorespiration and the Protection of Photosynthesis During Water Deficit. Plant Physiology, 150:1530-1540.

Ribeiro, R., Machado, R. S., Machado, E. C., Machado, D., Filho, J. and Landell, M. G. A. (2013). Revealing drought-resistance and productive patterns in sugarcane genotypes by evaluating both physiological responses and stalk yield. Experimental Agriculture, 49:02.

Rivero, R. M., Shulaev, V. and Blumwald, E. (2009). Cytokinin-Dependent Photorespiration and the Protection of Photosynthesis During Water Deficit. Plant Physiology, 150:1530-1540.

Robertson, M. J., Inman-Bamber, N. G., Muchow, R. C. and Wood, A. W. (1999). Physiology and productivity of sugarcane with early and mid-season water deficit. Field Crops Research, 64:211-227.

Sharma, V. (2009). Identiication of Drought-Related Quantitative Trait Loci (QTLs) in Sugarcane (*Saccharum* spp.) Using Genic Markers. DOCTOR OF PHILOSOPHY, Texas A&M University.

Silva, M. D. A.,. Jifon, J. L., Silva, J. A. G. D. and Sharma, V. (2007). Use of Physiological Parameters as Fast Tools to Screen for Drought Tolerance in Sugarcane. Plant Physiology, 19:193-201.

Singels, A. and Inman-bamber, N. G. (2002). The Response of Sugarcane to Water Stress: Preliminary Results from a Collaborative Project. Proceedings of the Annual Congress - South African. Sugar Technologists' Association, 240-244.

Singels, A., Kennedy, A. J.and Bezuidenhout, C. N. (2000). The Effect of Water Stress on Sugarcane Biomass Accumulation and Partitioning. Proceedings of the Annual Congress-South African Sugar Technologists' Association, 169-172.

Singels, A., Van den berg, M., Smit, M. A., Jones, M. R. and Van antwerpen, R. (2010). Modelling Water Uptake, Growth and Sucrose Accumulation of Sugarcane Subjected to Water Stress. Field Crops Research, 117:59-69.

Stiles, W., Monteith, J. L. and Bull, T. A. (1970). A diffusive resistance porometer for field use. Journal of Applied Ecology, 7:617-638.

Somkit, S., Vorasatit, N. and Ponluckdee, V. (2007). Study of Physiology and Botanical Characteristics of Drought Tolerant Sugarcane. Department of Agriculture, Ubon Ratchathani.

Tabkhkar, N., Rabiei, B., Samizadeh Lahiji, H. and Hosseini Chaleshtori, M. (2018). "Genetic Variation and Association Analysis of the SSR Markers Linked to the Major Drought-Yield QTLs of Rice." Biochem Genet, 56:356-374.

Tripathi, P., Chandra, A. and Prakash, J. (2019). Physio-biochemical assessment and expression analysis of genes associated with drought tolerance in sugarcane (*Saccharum* spp. hybrids) exposed to GA3 at grand growth stage. Plant Biology, 21:45-53.

Thai Encyclopedia for Youth. (1980). "Sugar cane growth." Retrieved from http://kanchanapisek.or.th/kp6/sub/book/book.php?book=5&chap=3&page=t5-3-info detail06. html.

Wilkinson, S., Kudoyarova, G. R, Veselov, D. S., Arkhipova, T. N and Davies, W. J. (2012). Plant Hormone Interactions: Innovative Targets for Crop Breeding and Management. Experimental Botany, 63:3499-3509.

Wang, Z., Li, G., Sun, H., Ma, L., Guo, Y., Zhao, Z., Gao, H. and Mei, L. (2018). Effects of drought stress on photosynthesis and photosynthetic electron transport chain in young apple tree leaves. Biology Open 7: bio035279.

(Received: 27 July 2019, accepted: 30 October 2019)