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Research Article
Classification of the Sugar Accumulation Patterns in Diverse Sugarcane Cultivars under Rain-fed Conditions in a Tropical Area

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Abstract

Background and Objective: Unsuitable sugarcane which immature or over-mature is imported to the mill, a critical problem, generally occurs in sugarcane production under tropical zone. Therefore, the objective of this study was to determine the sugar accumulation patterns of diverse sugarcane cultivars grown under natural rain-fed conditions in a tropical area. **Materials and Methods:** Randomized complete block design (RCBD) with 4 replications was used. Seventeen different elite lines, which represent all sugarcane production areas in Thailand, were used as treatments. Brix, commercial cane sugar (CCS) and fibre were collected during 8-15 months after planting (MAP). **Results:** The sugarcane lines used in these experiments can be classified into 6 groups, namely those that exhibit rapid sugar accumulation and CCS decreases with increasing temperature, rapid sugar accumulation and insensitive with temperature, medium sugar accumulation and decrease with temperature increasing, medium accumulation and insensitive, slow accumulation and decrease with increasing temperature and slow accumulation and insensitive. **Conclusion:** Seventeen sugarcane cultivars exhibited different sugar accumulation patterns, based on the speed of sugar accumulation and high temperature sensitivity at over-maturity.

Key words: Sucrose accumulation, brix, ripening stage, stalk diameter, rain-fed, tropical zone

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Sugarcane mainly grows in tropical and subtropical regions and most of the world's sugarcane is grown between 33°N and 33°S[1]. Cane production areas occupy several zones of the world, namely South America, South Asia, Africa, Australia, Polynesia and Melanesia. There are many countries that produce sugarcane, which is used as a raw material in sugar production, such as Brazil, China, India, Australia and Thailand. Sugarcane accounts for 80% of the total sugar production in the world[2]. Even though sugarcane is an important commercial crop, several problems occur in production and post-harvest processes[3]. A critical problem for sugar production commonly appeared in tropical zone is a variation of sugarcane which import to sugar mill, occurring immature or over-mature phases. This leads to low sugar content in cane stalk and low sugar yield via a short sugar accumulation period of the over-peak of sugar accumulation[4]. It seems likely that improving sugarcane breeding programs requires a sugarcane cultivar, which accumulates sugar rapidly and maintains sugar accumulation content as highly. Therefore, understanding sugar accumulation patterns of various genotypes is essential and needed for improving potential sugarcane cultivars, which have a wide range of harvesting. This would enhance sugar production in tropical areas.

At 9-11 MAP (270-330 days after planting), sugarcane initially accumulate sucrose in stalk, while the growth of sugarcane have plateaued. In the ripening phase, at 12 MAP, sugar accumulation of sugarcane is at its peak, however, this depends on genetic and environment factors, such as temperature, the photoperiod, soil moisture content and nutrients[5]. In the leaf organ of the sugarcane, the main function is sugar synthesis and sugars are not usually stored in the leaf. Disaccharides, namely sucrose are generated from two monosaccharides (glucose and fructose) and adenosine tri-phosphate (ATP) is used as supporting energy to transport these sugars to the phloem of the leaf and then the stalk. The transportation rate is dependent on genetics and environmental factors[6].

Cane yield and sucrose content are two important factors for obtaining high sugar yield[7,8]. Sucrose accumulation in developing sugarcane is accompanied by continuous synthesis and cleavage of sucrose in the storage tissues[9]. The major factors influencing polarity (Pol) bias are the sugar concentrations and their relative ratios[10]. Commercial cane sugar (CCS) is calculated from brix, Pol and the fibre content of the cane. CCS estimates the level of extractable sucrose based on the level of impurities at harvest[10]. Sugarcane ripening is the process of sucrose accumulation in stalks, which is heavily influenced by several factors, mainly climatic conditions, such as air temperature and water deficits[5]. Temperatures can fluctuate rapidly during the sugarcane harvest season but the effect of short-term temperature on sugar metabolism in sugarcane internodes is unknown[11]. Sugar concentration in sugarcane is influenced by the balance of sucrose metabolism enzymes in the stem[11-13]. Stalk death reduces dry matter and sucrose accumulation[14].

Despite many previous reports revealing sugar accumulation mechanisms and factors associated with sucrose accumulation, sugar accumulation patterns are still poorly understood and there is no information of various sugarcane cultivars and environments. Therefore, the objective of this study was to determine the sugar accumulation patterns of diverse sugarcane cultivars grown under natural rain-fed conditions in tropical areas. This information can be used to support breeding programs as a selection criterion for improving sugar yield.

MATERIALS AND METHODS

Experimental details: Two field experiments were conducted in two locations, namely (1) The Field Crop Research Station of Khon Kaen University, Khon Kaen province, Thailand, from November, 2013 to February, 2015 and (2) Farmer field at Nong Sang district, Udon Thani province, Thailand, from December, 2013 to February, 2015. The first field consisted of a plot of 5 rows, 8 m long, with a spacing of 1.65 m between rows. The soil type was Yasothon soil that has physical properties including 87.48% sand, 8.81% silt and 3.71% clay, making it defined as a sandy soil. The second field test consisted of a plot of 4 rows, 8 m long, with a spacing of 1.65 m between rows, which there was Sa-tuk soil series that it had physical properties including 84.72% sand, 9.57% silt and 5.51% clay, defining it was loamy sand soil. Randomized complete block design (RCBD) with four replications was used.

Seventeen different elite lines, namely KKU99-01, KKU99-02, KKU99-03, KKU99-06, TBy28-0941, TBy28-1211, Kps01-12, K88-92, CSB07-79, CSB07-219, UT12, UT13, KK3, KK06-419, KK06-501, MPT05-187 and MPT02-458, which represent all sugarcane production areas in Thailand, were used as treatments. These sugarcane genotypes were crossed and selected from Khon Kaen University (KKU99-01, KKU99-02, KKU99-03 and KKU99-06), which selected for drought conditions. TBy28-0941, TBy28-1211 and Kps01-12 were derived from Kasetsart University, which crossed and selected in the centre part of Thailand sugar production area. K88-92, CSB07-79 and CSB07-219 were derived from the Office of Cane and Sugar Board, Thailand. KK3, KK06-419 and KK06-501 were derived from the Department of Agriculture, Thailand, which improves cane genotypes for drought resistance. MPT05-187 and MPT02-458 were elite line from breeding program for high yielding of Mitr Phol Company.

Crop management: Cane stalk of each cultivar were selected if they had no diseases, infections or insect destruction. Chopped cane stalk about 3-4 buds per sett were planted in the field. For both locations, basal fertilizers were applied to the field at a rate of 132.5 kg ha^{-1} (19.9 kg N, 19.9 kg P and 19.9 kg K), which these experiment under rain-fed conditions. At 4-5 MAP top dressing fertilizer was applied at a rate of 132.5 kg ha^{-1} (6.5 kg N, 6.5 kg P and 10.5 kg K). Weed control was done 2-3 MAP and herbicide was sprayed at a rate of 400 mL per 60 L amount of water (H_2O) in 6-7 MAP. Insect, disease controls were conducted as imperative to keep the plants free from pets throughout the course of the experiment.

Data collection

Weather data: Meteorological data were daily collected. Rainfall and maximum and minimum temperatures were recorded using the information from the weather station at Northeastern meteorological centre, Thai Meteorological Department (TMD). The weather data derived from TMD were obtained separately for Khon Kaen and Udon Thani provinces. The Khon Kaen weather station was located 5 km from the first experimental field and the second field test was located 36 km from the weather station.

Agronomic characteristics: In both fields, stalk diameter (mm) was monthly measured for four main stalks per plot 8-9 MAP using digital calipers (Vernier). Green leaf numbers were counted at the main stem of the plant, recording four stalks per plot 1 month intervals during 8-9 MAP.

Brix, commercial cane sugar (CCS) and sugar yield data: Brix was collected on the juice using an automatic temperature compensated refractometer (20°C) (digital brix refractometer, ATAGO PAL 1), measuring from four main stalks per plot. Brix of 17 sugarcane cultivars were monthly recorded during 8-15 MAP in Khon Kaen experiment, while Udon Thani during 8-12 MAP.

CCS was monthly determined (during 12-15 MAP in Khon Kaen experiment and during 12-14 MAP in Udon Thani experiment) for 26.4 m^2 harvesting areas, using random of 6 stalks from all stalks in harvesting areas[15]. For the determination of pol, 2.5 g of lead acetate was thoroughly mixed with the juice, which was then filtered through Whatman No. 91 filter paper and the filtrate was passed through a polarimeter (Polartronic NIR W2, Schmidt and Haensch, Berlin, Germany). The fresh weight and dry weight of the remaining stalk material were determined for calculation of the fibre content. The dry weight of sugar yield per plot was then calculated based on adjusted CCS values as follows[15-17]:

$$\text{CCS} = \frac{3}{2}\text{P}\left(1-\frac{\text{F}+5}{100}\right)-\frac{\text{B}}{2}\left(1-\frac{\text{F}+3}{100}\right) \quad (1)$$

where, P = pol at 20°C, B = brix at 20°C and F = fibre percent. Cane yield (t ha^{-1}) was measured at 12 MAP. Harvest are an occupied of the centre two rows with 8 m long total around 26.4 m^2 in each plot. Sugarcane was collected six tillers per plot, which removed leaves and the top of stalk. Sample was then fresh weight to calculate for yield per area. Sugar yield at 12 MAP was then calculated based on cane yield and CCS values as follows[15-17]:

$$\text{Sugar yield} = \frac{\text{Cane yield}\times\text{CCS}}{100} \quad (2)$$

Statistical analysis: Statistical analysis was conducted using Statistix 8. The measured data were subjected to analysis of variance according to a RCBD. The comparison among genotypes for all parameters was done based on the Least Significant Difference (LSD) test[18]. Simple correlation was used to determine the correlation between growths and sugar accumulation. Stalk diameter, number of green leaves, stalk height and brix data were calculated into the different value between 8 and 9 MAP as mention below[19]:

$$\text{Change of value} = (\text{value* of 9 MAP})\text{-}(\text{value* of 8 MAP}) \quad (3)$$

where, * = Values of stalk diameter/number of green leaves/brix.

RESULTS

Climatic data during the sugarcane growth period: In Khon Kaen Province, the maximum daily air temperature average was 32.15°C, the minimum temperature average was 21.59°C and the total rainfall was 1,048 mm during the sugarcane growth period. In the sugar accumulation stage at 8-15 MAP (July, 2014 to February, 2015), the monthly average temperatures were 29.0, 28.6, 28.4, 27.8, 27.3, 23.9, 21.4 and 25.4°C, respectively and total rainfall was 155.2, 200.6, 257.9,

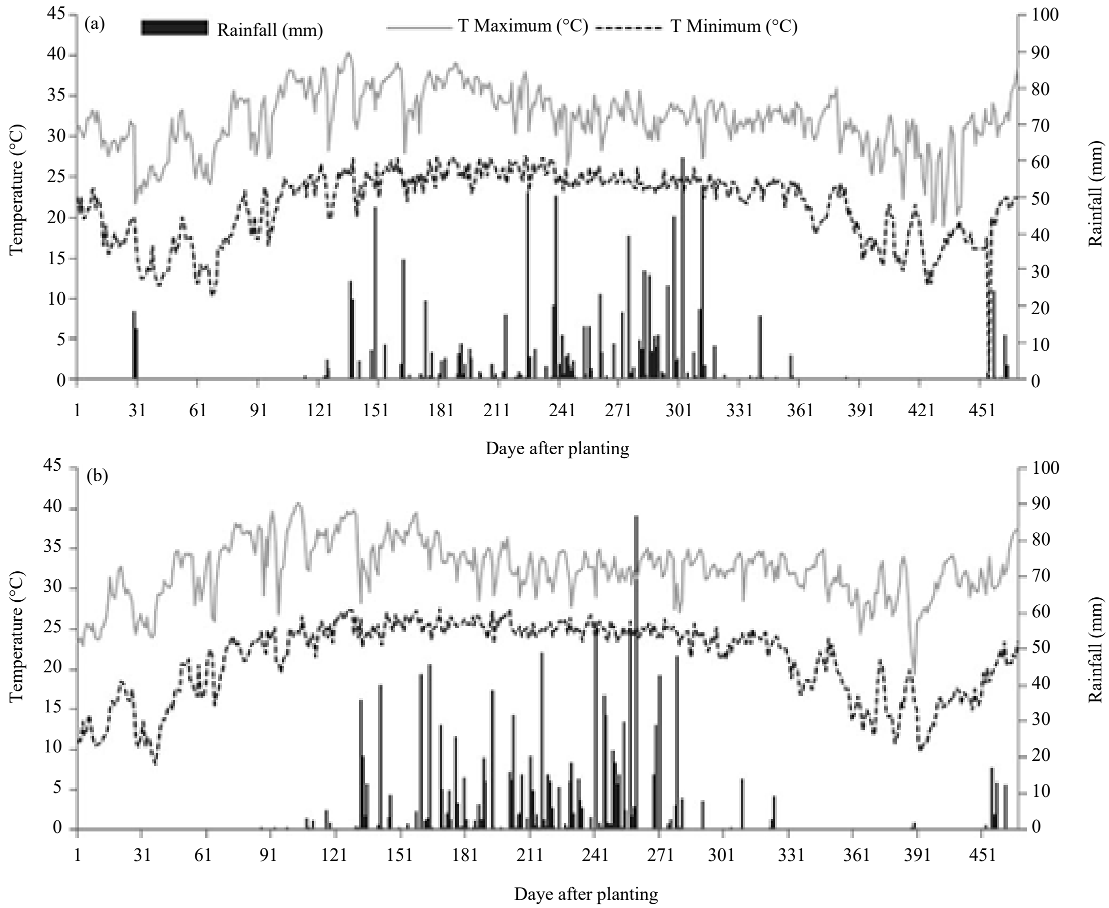

Fig. 1(a-b): Rainfall (mm), maximum temperature (°C) and minimum temperature (°C) during the sugarcane growth period derived from the metrological station in the (a) Khon Kaen province (November, 2013 to February, 2015) and (b) Udon Thani Province (December, 2013 to February, 2015)

19.3, 0.1, 0.0 and 42.4 mm, respectively (Fig. 1a). In Udon Thani province, the maximum daily air temperature average was 32.42°C, the minimum temperature average was 21.30°C and the total rainfall was 1.357 mm during the sugarcane growth period. In the sugar accumulation stage at 8-14 MAP (August, 2014 to February, 2015), the monthly average temperatures were 28.7, 28.6, 28.2, 26.9, 23.2, 22.1 and 25.4°C, respectively and the total rainfall was 303.4, 307.3, 21.9, 12.8, 0.0, 2.4 and 47.6 mm, respectively (Fig. 1b).

Sugar accumulation patterns: Brix data from 8-10 MAP was used for determining early sugar accumulation during the harvesting stage and 12-15 MAP of CCS set data were used to indicate a sensitivity of sugar accumulation to high temperature. The sugar accumulation patterns between both locations were different and were exhibited separately.

In Khon Kaen, KK3, KKU99-01 Kps01-12, MPT02-458 and KK06-501 were identified as rapid sugar accumulation (compared with the mean of all genotypes) base on brix data from 8-10 MAP (Fig. 2). Medium sugar accumulation occurred in TBy28-0941, UT13, TBy28-1211, CSB07-79, KKU99-02 and MPT02-187, whereas K88-92, KK06-419, UT12, CSB07-219, KKU99-03 and KKU99-06 were slow accumulation (Fig. 2). For CCS value, KK3, KKU99-01, TBy28-0941, UT13, K88-92 and KK06-419 revealed decreased with increasing temperature from 14-15 MAP but Kps01-12, MPT02-458 and KK06-501 TBy28-1211, CSB07-79, KKU99-02 and MPT02-187 UT12, CSB07-219, KKU99-03 and KKU99-06 were insensitive (Fig. 3). Therefore, the sugarcane lines used in these experiments can classified into six groups, namely (1) Those that exhibit rapid sugar accumulation and CCS decreases with increasing temperature (KK3 and KKU99-01), (2) Rapid sugar accumulation and CCS value insensitive with increasing temperature (Kps01-12, MPT02-458 and KK06-501), (3) Medium sugar accumulation and CCS value decrease with increasing temperature (TBy28-0941 and UT13), (4) medium

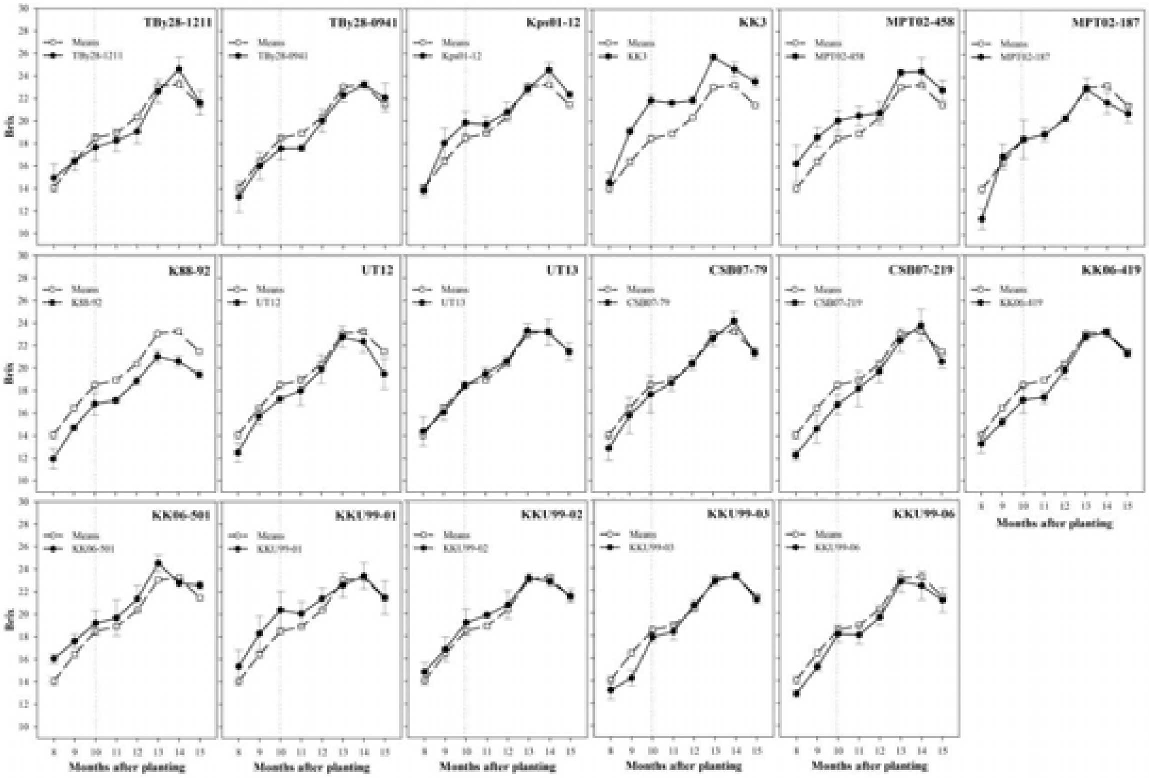

Fig. 2: Brix values of 17 sugarcane genotypes during 8-15 months after planting in the Khon Kaen Province

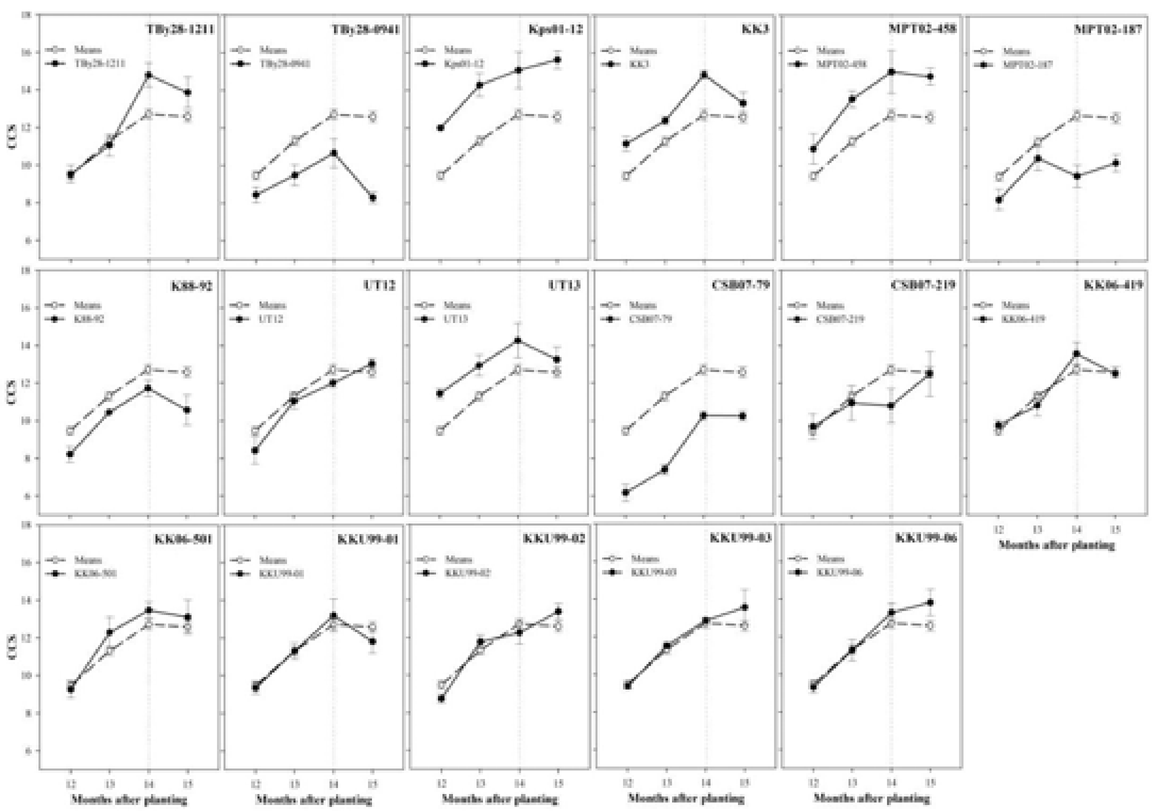

Fig. 3: Commercial cane sugar (CCS) of 17 sugarcane genotypes during 12-15 months after planting in the Khon Kaen Province

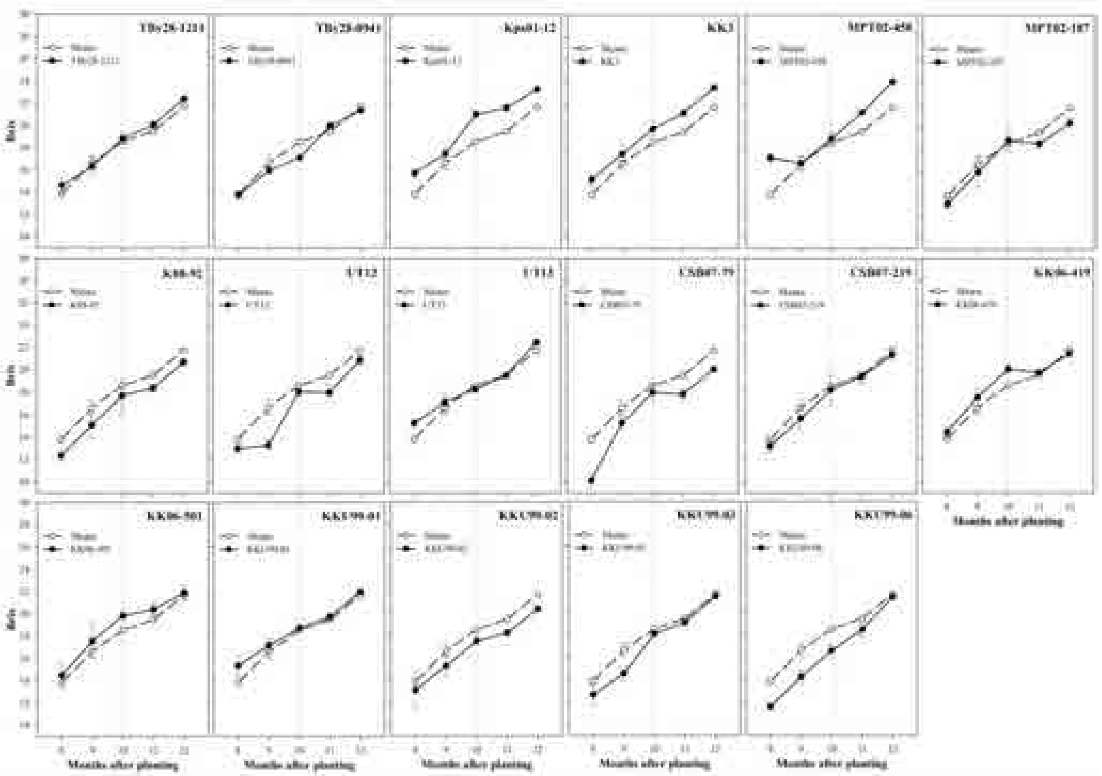

Fig. 4: Brix values of 17 sugarcane genotypes during 8-12 months after planting in the Udon Thani Province

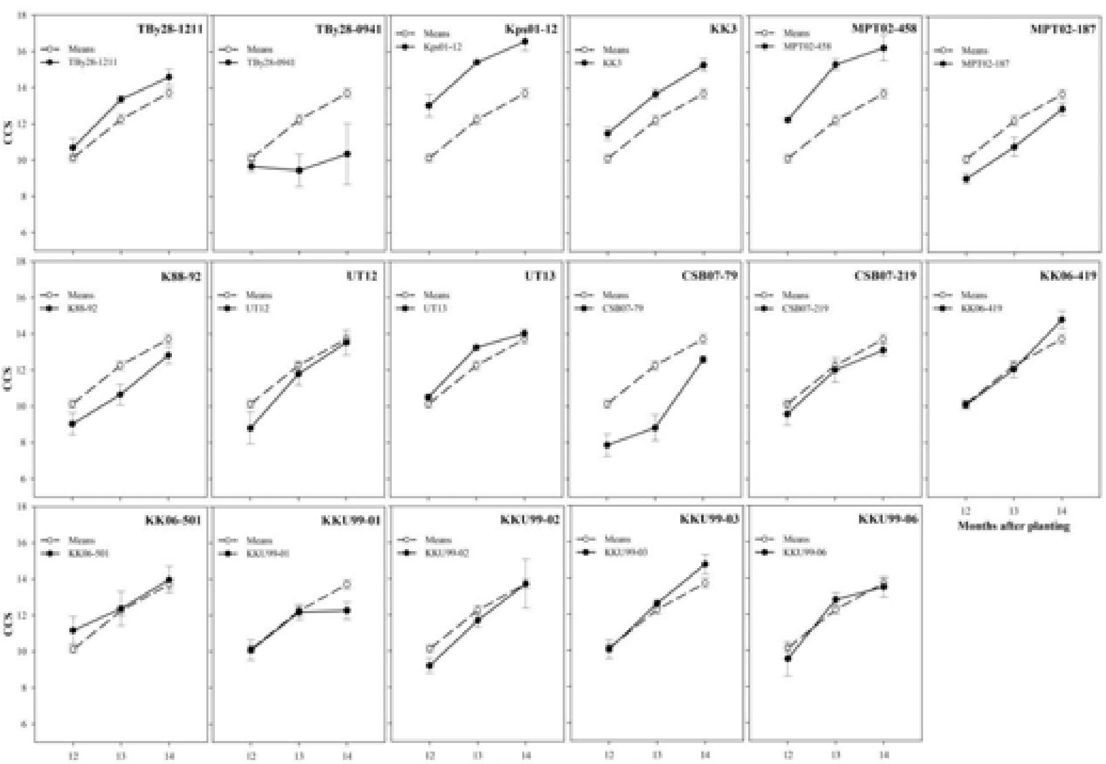

Fig. 5: Commercial cane sugar (CCS) of 17 sugarcane genotypes during 12-14 months after planting in the Udon Thani Province

sugar accumulation and CCS value insensitive with increasing temperature (TBy28-1211, CSB07-79, KKU99-02 and MPT02-187), 5) slow sugar accumulation and CCS value decrease with increasing temperature (K88-92 and KK06-419) and (6) slow sugar accumulation and CCS value insensitive with increasing temperature (UT12, CSB07-219, KKU99-03 and KKU99-06).

In Udon Thani, KK3, Kps01-12, KK06-501 and KK06-419 were identified as rapid sugar accumulation and medium group including TBy28-1211, TBy28-0941, MPT02-458, MPT02-458, CBS27-79, CSB07-219, KKU99-01 and UT13 (Fig. 4). The remaining lines were grouped as the third one (Fig. 4). All genotypes revealed CCS value insensitive with increasing temperature (from 13-14 MAP) (Fig. 5).

For magnitude of sugar contents, based on the consistency of CCS value in 12-14 MAP for both locations, KK3, Kps01-12, MPT02-458 and UT13 were identified as a higher CCS value (Fig. 3 and 5). In contrast, lower CCS values were revealed in TBy28-0941, MPT02-187, K88-92 and CSB07-79 (Fig. 3 and 5). Thus, the cultivar with potential for sugar accumulation was Kps01-12, provided early accumulation of sugar, higher sucrose content and insensitivity to high temperature at over-maturity. In addition, varieties KK3 was suitable for early harvesting and MPT02-458 suitable for late cutting.

Relationship between sugar accumulation and fibre patterns: All sugarcane genotypes tend to reduce fibre content after the start of the sugar accumulation stage in Khon Kaen experiment, whereas CCS patterns gradually increased at 12-13 MAP (Fig. 6). In addition, a particular genotype showed increased fibre content when the CCS value was reduced via higher temperature at 14-15 MAP, except for Kps01-12, MPT02-187, UT12, CSB07-219, KKU99-02, KKU99-03 and KKU99-06 had increased for both values (Fig. 6). In Udon Thani, UT12, CSB07-79, KKU99-02 and KK06-419 showed decreased fibre content when the CCS value was increased via higher temperature at 13-14 MAP (Fig. 7).

In both locations, there was no correlation between brix and stalk diameter and green leaves number and height of all sugarcane cultivars (data not shown). However, in term of the difference of values between 8 and 9 MAP, the correlation between brix and stalk diameter showed negative relationship

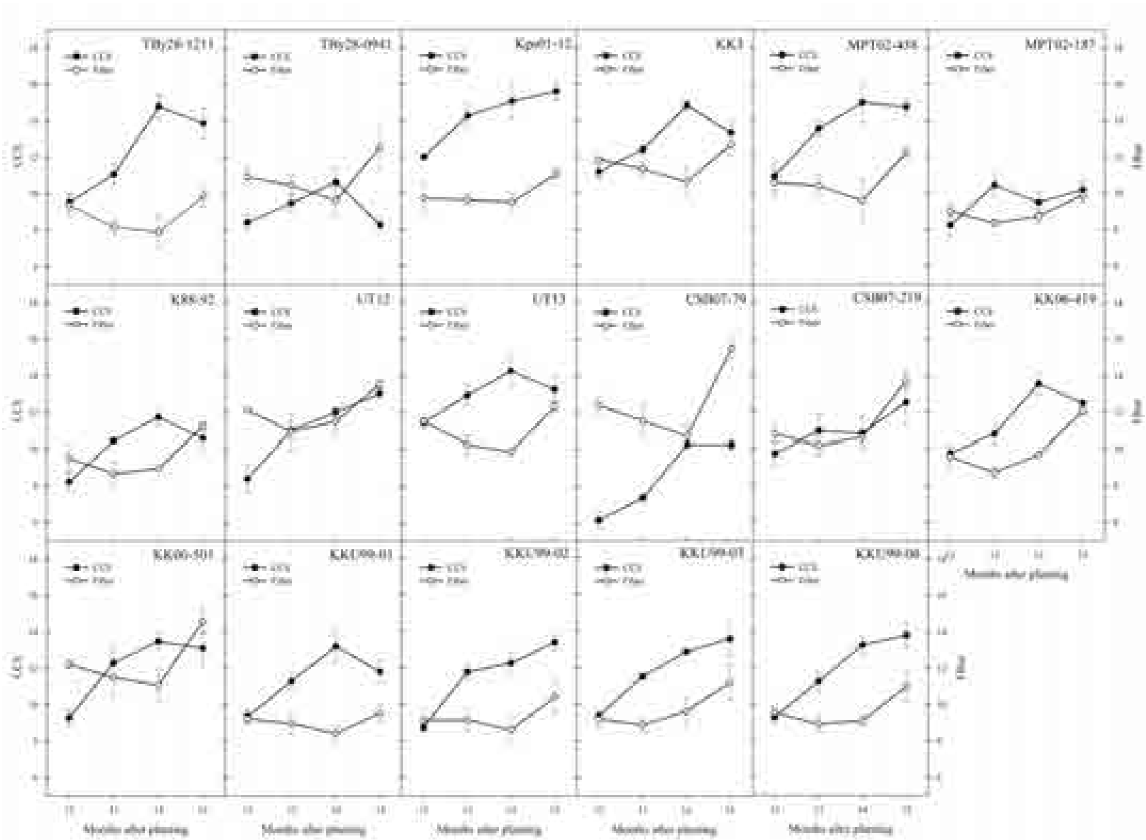

Fig. 6: Changes in CCS values and fibre content of 17 sugarcane genotypes during 12-15 months after planting in the Khon Kaen Province

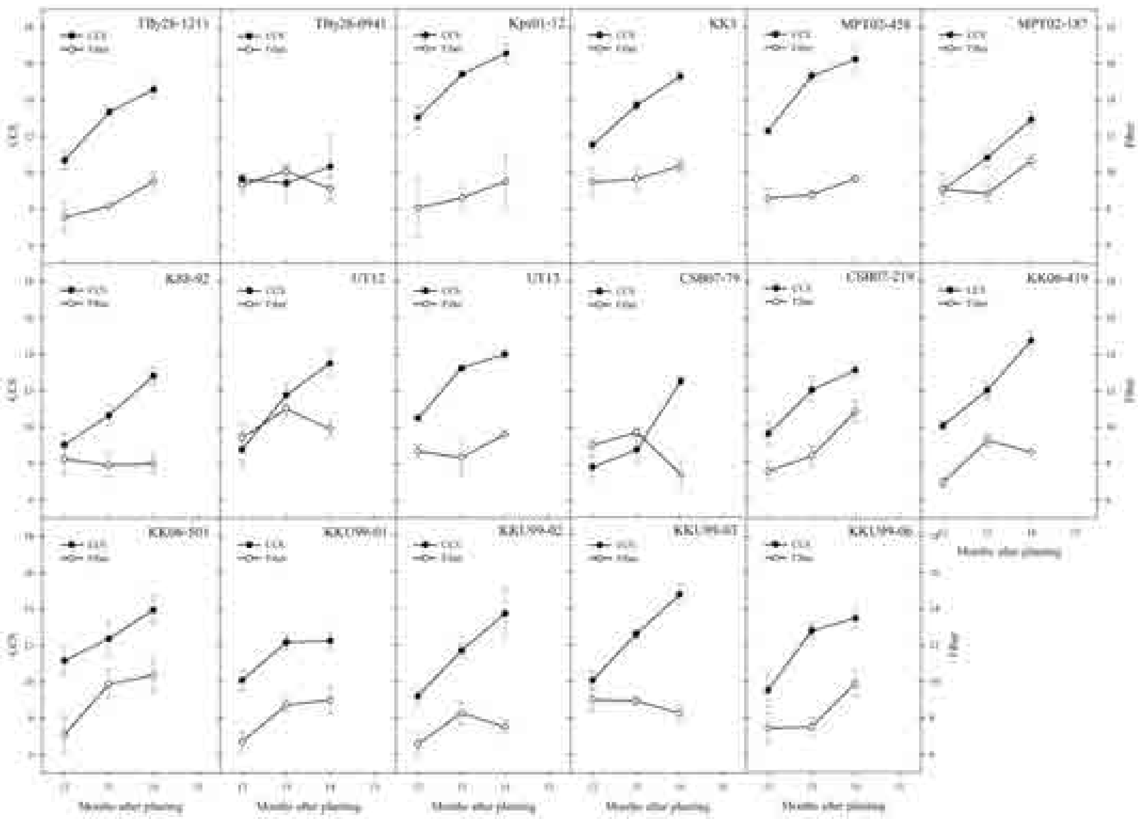

Fig. 7: Changes in CCS values and fibre content of 17 sugarcane genotypes during 12-14 months after planting in the Udon Thani Province

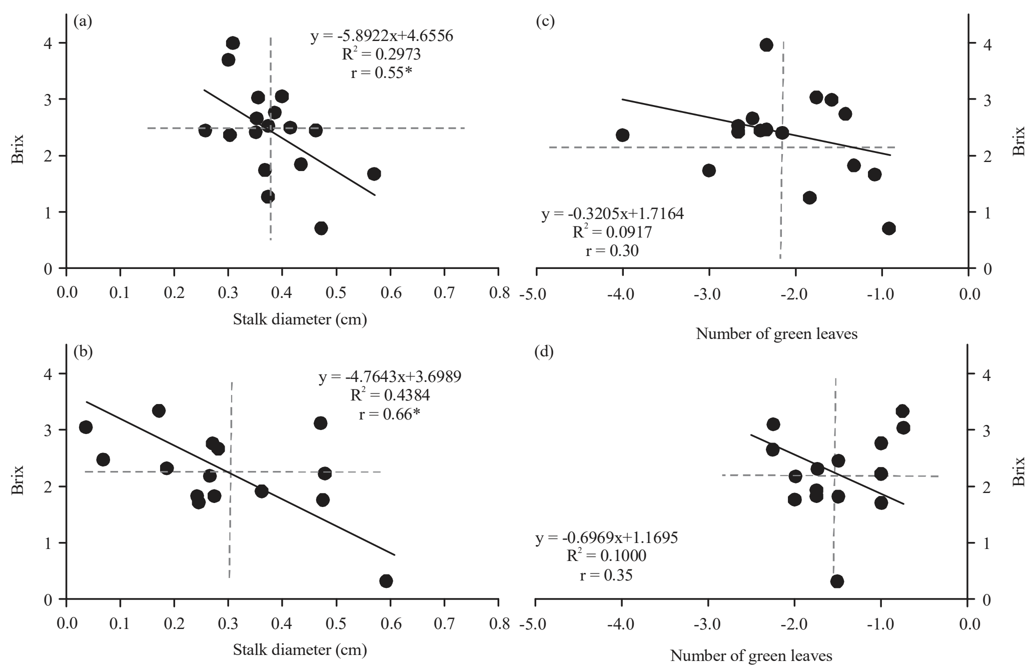

Fig. 8(a-d): Correlation (n =17) between the change in (a, b) Brix and stalk diameter (cm) from 8-9 months after planting and (c, d) Brix and number of green leaves of 17 sugarcane genotypes in Khon Kaen (a, c) and Udon Thani (b, d) Provinces
*Significant at 0.05 probability levels

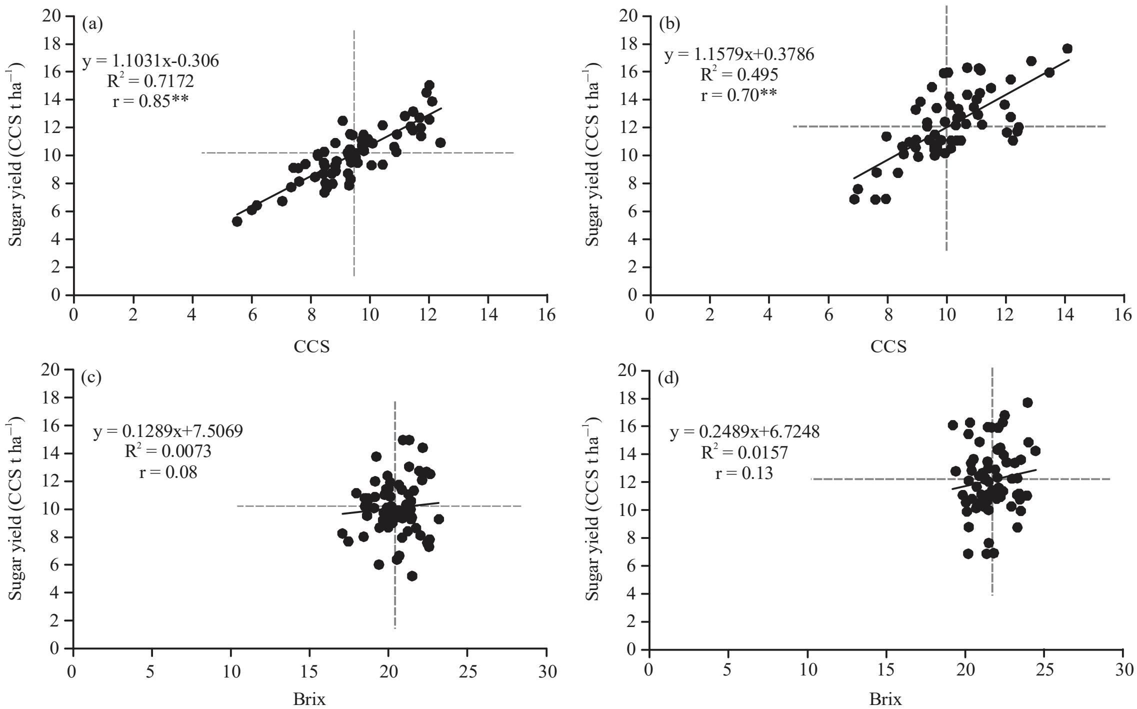

Fig. 9(a-d): Correlation coefficients (n = 68) between (a, b) Sugar yield (CCS t ha^{-1}) and commercial cane sugar (CCS), (c, d) Sugar yield (CCS t ha^{-1}) and brix of 17 sugarcane genotypes during 12 months after planting in Khon Kaen (a, c) and Udon Thani (b, d) Provinces

**Significant at 0.01 probability levels

in Khon Kaen and Udon Thani experiments (-0.55 and -0.66, respectively) (Fig. 8a, b), indicating stalk diameter decreased after the start of the sugar accumulation stage. There was no relationship between the difference of brix values and green leaves number (Fig. 8c, d).

Relationship between sugar accumulation and growth: In Khon Kaen and Udon Thani Provinces, the sugar yields of all sugarcane cultivars were positively related with CCS (Fig. 9a, b), whereas the sugar yield was not related with brix (Fig. 9c, d). In addition, in Khon Kaen province sugar yield and all shoot characteristics (tiller number, stalk length and biomass) did not correlate (Fig. 10a-c). In Udon Thani, 17 sugarcane cultivars had positive correlations between sugar yield and shoot traits, such as tiller number and biomass (Fig. 10d, e) but there were no relationships with stalk length (Fig. 10f).

DISCUSSION

Sucrose accumulation in stalks is a key activity of sugarcane in repining stage, which is seriously affected by several factors[5,20]. The synthesis of sucrose is a complex mechanism that involves with genetic, enzyme, developmental stage, field management and environment[21]. For this result, the responses of CCS value with increasing temperature at ripening stage of two site experiments were different. Due to being in the developmental stage, younger cane in Udon Thani was insensitive to higher temperatures. Therefore, the appropriate conditions, together with the developmental stage and genetics are key to determining the sugar storage of sugarcane[5,21]. The dry season and low temperatures in the humid-tropical and subtropical regions slow down the growth of sugarcane, forcing the conversion of reducing sugar to sucrose[4]. The high sugar content of sugarcane occurs when there is low air temperature for a long period of time[22]. Moreover, high air temperature and soil moisture content can change invertase balance in intense growth, decreased non-reducing sugar accumulation and slow down ripening[5].

As the sugar accumulation patterns, CCS value in this study gradually increased from 8-12 MAP. This conforms with previous literature stating that the sucrose concentration drastically increases from 9-12 MAP, with an 8% increase in sucrose concentration[5]. Despite the developmental stage of sugarcane being a main factor to control sugar accumulation,

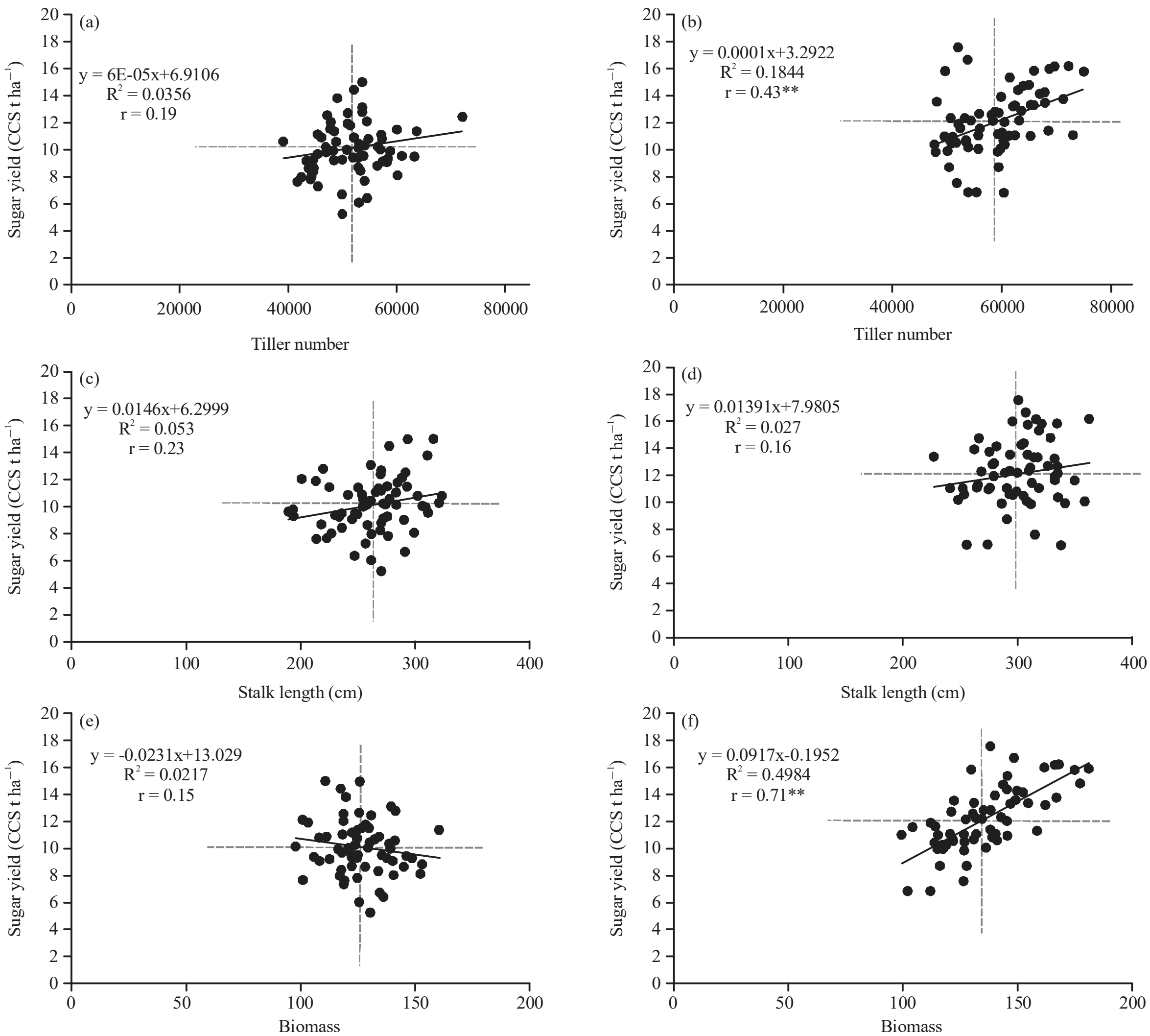

Fig. 10(a-f): Correlation coefficients (n = 68) between (a, b) Sugar yield (CCS t ha^{-1}) and tiller number (c, d) Stalk length and (d, e) Biomass of 17 sugarcane genotypes during 12 months after planting in Khon Kaen (a, c, e) and Udon Thani (b, d, f) Provinces

**Significant at 0.01 probability levels

genetic variations can create deviations in sugar storage behaviours. The precocity of cultivar can actually generate a difference in sugar yield[23]. Early sugar accumulation cultivar are sensitive to weather conditions for ripening, while the late cultivars are less sensitive[24], due to enzyme activity. Invertases functions to provide growing tissue with monosaccharide as a source of energy[25-27]. The reactions of invertase activity and sugar levels are in relation, early genotypes tend to have higher neutral invertase activity levels earlier than late genotypes[5].

Ripening of sugarcane is due to a complex combination of climate, genetics and agricultural management[5]. Changes inside the plant involve ripening responses. Through the ripening period, sucrose concentrations along the entire stalk increase and the proportion of the stalk containing significant glucose and fructose concentrations decreases[28]. Sucrose concentration was negatively correlated with soluble acid invertase[27-29], therefore, sucrose accumulation in the stalk decreased when soluble acid invertase increased[13,28,30]. Soluble acid invertase played a critical role in limiting sucrose accumulation in the 9 month old sugarcane. Invertase activity had a negative hyperbolic relationship with sucrose concentration[13,28]. Sucrose concentration, which is represented by the CCS value, is dependent on its net result of synthesis and breakdown[31-33]. Hence, Biomass production and sucrose concentration are interlinked processes that are involved in the sucrose content in the stem of sugarcane[34]. Mature internodes have higher sucrose content, while higher monosaccharide and lower sucrose concentrations occur in younger internodes[7].

Sugarcanes tend to reduce fibre content after the start of the sugar accumulation stage. Fibre is the dry and water-insoluble component of the stalk[35]. Sugar accumulation patterns of individual stalk depend on the fibre pattern, which exist at low CCS values and at high fibre fractions[14]. The morphology of the sugarcane can lead to qualitative and quantitative changes in production via supporting decrease in growth and increment of non-reducing sugar content and the sugar yield[36], which depends on biomass and sucrose content[34]. In the harvest period, non-reducing sugar levels in the stem gradually increase, whereas the percentages of reducing sugar decrease[4]. Growth rates of stalk, either in diameter or length are reduced via enhancement of sugar accumulation rates[37] and a lower sugar content is also as result of new tissue formation[5].

In addition, Cardozo and Sentelhas[5] also grouped the sugarcane cultivars based on the variation of sugar content decrease into early middle and late cultivars. In this study, the cultivar with potential for sugar accumulation, such as Kps01-12, provided early accumulation of sugar, higher sucrose content and insensitivity to high temperature at over-maturity. In addition, varieties KK3 was cultivars which appropriate with early ripening and MPT02-458 cultivar suitable for late harvesting.

The improvement of sugar productivity need to concern a suitable cultivar corresponded with harvesting conditions. Early sugar accumulation cultivar should be harvested as a prior and then the late one. Moreover, this information can be used to support breeding programs as a selection criterion for improving sugar yield.

CONCLUSION

Seventeen sugarcane lines used in these experiments can be classified into 6 groups, based on the speed of sugar accumulation and high temperature sensitivity at over-maturity. Moreover, negative relationship between brix and stalk diameter was existed, larger stalk did not support to high brix value. This information can be used to support breeding programs as a selection criterion for sugar yield, improving with different cutting times, such as early, middle and late crop harvests.

SIGNIFICANCE STATEMENT

This study discovers different sugar accumulation patterns with diverse sugarcane cultivars that can be beneficial for supporting breeding programs as a selection criterion for sugar yield. This study will help the researcher to uncover the critical area of sugar accumulation patterns under natural rain-fed conditions that many researchers were not able to explore. Thus, a new category on these patterns, improving with different cutting times, may be arrived at.

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