Article

Association of Physiological Responses and Root Distribution Patterns to Ratooning Ability and Yield of the Second Ratoon Cane in Sugarcane Elite Clone

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Abstract: The objective of this study was to determine the association of physiological responses and root distribution patterns on yield of the second ratoon cane and the relationships among these traits. Seventeen sugarcane genotypes were planted in a randomized complete block design with four replications. The second ratoon crop was evaluated for germination percentage, cane yield, SPAD chlorophyll meter reading (SCMR), chlorophyll fluorescence, relative water content (RWC), specific leaf area (SLA) and stomatal conductance. Root length density (RLD) was evaluated by auger method. The root samples were divided into upper soil layer and lowers soil layers to study root distribution patterns. Sugarcane genotypes were significantly different for RLD, germination percentage and cane yield. Root distribution patterns were classified into three groups based on the RLD. High RLD between plants in the upper soil layers at 90 DAH was positively correlated with high germination, whereas high RLD between rows in the lower soil layers at 90 and 270 DAH was associated with high cane yield. RWC at 90 DAH and stomatal conductance at 180 DAH were closely related to germination percentage, whereas chlorophyll fluorescence and stomatal conductance at 180 DAH were closely related to cane yield.

Keywords: root length density; ratoon crop; *Sacharum* spp., varietal selection

1. Introduction

Sugarcane is an industrial crop and used mainly as a raw material for sugar production. The byproducts from sugar mill are also used for production of other value-added products such as electricity, veneer, monosodium glutamate and ethanol. Sugarcane is largely grown under rain-fed conditions in arid and semi-arid tropic regions.

Drought stress is the most important factor reducing rationing ability and sugarcane production in the world. Reduction in cane yield has been estimated at up to 60 percent [1]. Low yield of ration crop is mainly due to low and differential rationing potential of cultivars and suboptimal crop management. Ration crop has lower production cost than planted crop by 25-30 percent [2].

Sugarcane varieties respond differently to drought stress for sprouting, millable canes and commercial cane sugar and ratooning ability [3]. Imprudent of ratooning ability in sugarcane will not be possible if the mechanism underlying the maintenance of ratoon is not fully understood. Therefore, a better understanding on the association of physiological traits of shoot and root in maintaining higher ratooning ability should improve the efficiency of sugarcane production and breeding programs.

Root is an important plant part that takes up nutrients and water for growth and yield of crops. Sugarcane can develop new root system within three days after harvest to replace the old root system. New roots emerged from the basal nodes of young shoots over the next several weeks, but, at 7 weeks after harvest, a few new roots emerged from old roots that had been active at the time of harvest [4].

Drought reduced root growth in upper soil layers and root growth is shifted toward the lower soil layers to maintain water uptake [5].

In general, root system and physiological traits of shoot may be inter-related, and the developmental stage of root strongly contributes to the total above-ground growth of plants [6]. (Physiological traits related to photosynthesis such PSII photochemical efficiency (Fv/Fm), stomatal conductance, transpiration, SPAD index and water potential were identified as the traits promoting photosynthesis of sugarcane [7-9].

The information on the responses to drought for physiological traits related to germination and yield of different sugarcane genotypes is still lacking. The objective of this study was to determine the association of physiological responses and root distribution patterns on yield of the second ratoon cane and the relationships among these traits. The information obtained in this study will be useful in explaining the association between ratooning ability and root distribution patterns for recommendation of the surrogate traits for improving sugarcane genotypes in breeding programs.

2. Materials and methods

2.1. Experimental design and plant materials

Seventeen sugarcane genotypes consisting of 14 elite clones (KKU99-02, KK06-419, MPT02-458, UT13, KKU99-01, KKU99-06, KKU99-03, CSB07-219, CSB07-79, TBy28-1211, KK06-501, MPT05-187, UT12 and TBy28-0941) and three check varieties (Kps01-12, KK3 and K88-92) were laid out in a randomized complete block design with four replications at the Faculty of Agriculture, Khon Kean University, Thailand, to evaluate ratooning ability and yield of the second ratoon cane during the growing season 2016.

The cane stalks were cut into single node segments and the single node segments were planted directly in the five-row plots with 8.0 m long and 1.5 m distance between rows in November 2014. The spacing used was 1.5 m between row and 1.0 m within row. Data were recorded from 3 middle rows, discarding the plants at the ends of the rows. The planted crop and the first ratoon crop were harvested in 2014 and 2015, respectively. Chemical fertilizer formula 15-15-15 of N-P2O5-KO2 (Thai Central Chemical Public Company Limited, Phra Nakhon Si Ayutthaya, Thailand) at the rate of 312. 5 kg./ha was applied to the ratoon crop at two splits. The first split was applied after the first ratoon crop was harvest and the second split was applied at tillering stage (about 4 months after harvest). Weed control was carried out manually at 4 months after harvest of the first ratoon crop.

2.2. Data collection

2.2.1. Meteorological and soil data

The meteorological data were recorded during January–December 2016. Rainfall, maximum and minimum temperatures were recorded by the weather station at Agronomy field crop, Faculty of Agriculture, Khon Kaen University. The data of soil moisture content data were recorded at 90,180 and 270 days after harvest (DAH) at depth of 0–20, 20-40, 40-60, 60-80 and 80-100 cm. The soil samples were weighed and oven-dried at 105 °C for 72 hours. Thereafter, the percentage of soil moisture was determined from weights of wet soil and dry soil.

2.2.2. Germination percentage

Number of plants was recorded at 60 days after harvest (DAH) of the first ration crop, and germination percentage was calculated as follows;

2.2.3. Physiological traits

Physiological traits consisting of specific leaf area (SLA), SPAD chlorophyll meter reading (SCMR), chlorophyll fluorescence, stomatal conductance and relative water content (RWC) were measured at 90, 180 and 270 DAH from the second fully expanded leaf of the main stalk during 9.00-12.00 a.m. Leaf area was measured using LI-3100C Area Meter, LI-COR, inc. Lincoln, Nebraska USA. Leaf samples were oven-dried at 80 °C for 72 hr. or until the weights were constant and the dry weights were measured. SLA was calculated as the ratio between leaf area (cm²) and leaf dry weight (g). SPAD chlorophyll meter reading (SCMR) was measured using SPAD chlorophyll meter (SPAD-501, Minolta, Tokyo, Japan), at bottom, middle, and tip of the leaf. Chlorophyll fluorescence was measured on intact leaves using Chlorophyll fluorescence (MINI PAM, Heinz Walz GmbH, Germany) at 6.00-8.00 p.m. Stomatal conductance was measured by SC-1 Leaf Porometer (Decagon Devices, inc. WA 99163, USA). Relative water content (RWC) was measured from the weight difference between freshly harvested leaf and water saturated leaf. Leaf samples were imbibed in distilled water for 24 hours. The water saturated leaves were dried with blotting paper to eliminate the excessive water. Leaf saturated weight was then determined. The leaf samples were then ovendried at 80 °C for 72 hours and leaf dry weight was measured. Then RWC was calculated as follows;

$$RWC = \left(\frac{\text{(Leaf fresh weight - Leaf dry weight)}}{\text{(Leaf saturated weight - Leaf dry weight)}}\right) \times 100$$

2.2.4. Root traits

Root length density (RLD) was measured at 90 and 270 DAH using an auger method. The auger consisted of a coring tube [10] with a diameter of 69 mm. and a length of 1.15 m. The auger was designed to reduce compaction in the inner tube by improving the cutting edge and reducing the tube thickness [10,11]. Root samples were collected from two positions including the position between plant at a distance of 25.0 cm and the position between row at a distance of 75.0 cm. Root samples were taken to a depth of 100 cm and separated into five layers consisting of 0–20, 20-40, 40-60, 60-80 and 80-100 cm.

Root samples of each layer were washed manually with tap water to remove soil from the roots. The root samples were then analyzed with the Winrhizo program (Winrhizo Pro (s) V. 2004a, Regent Instruments, Inc) to determine root length. RLD was calculated as the ratio between root length (cm) and soil volume (cm³). RLD from the first (0–20 cm.) and second (20-40 cm.) layers were combined and defined as a upper soil layer (0–40 cm.), while the RLD for the deeper layers (third to fifth) were combined to a single 40-100 cm. layer as lower soil layer.

2.2.5. Yield

The cane productivity was calculated from the weight of all millable canes per plot and the area occupied by each plot.

2.3. Statistical analysis

Data were subjected to analysis of variance (ANOVA) according to a randomized complete block design. Means were separated by least significant difference (LSD) at 0.05 probability level. Simple correlation was used to determine the relationships among physiological traits, ratooning ability and cane yield.

3. Results and Discussion

3.1. Meteorological conditions and soil moisture content

This experiment was conducted during January to December 2016. Maximum temperature and minimum temperature were 34.0 and 22.6 °C, respectively (Figure 1). Total rainfall during the experimental period was 1,119 mm. Soil moisture contents at 90 (drought period), 180 (recovery period) and 270 DAH were clearly different (Figure 2). At 90 and 180 DAH, soil moisture contents in

top soil were lower than in sub soil (Figure. 2a, 2b). At 270 DAH, soil moisture contents in top soil were higher than sub soil (Figure 2c).

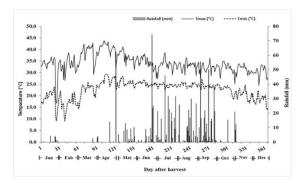


Figure 1. Rainfall (mm), maximum temperature (Tmax) and minimum temperature (Tmin) during January–December 2016.

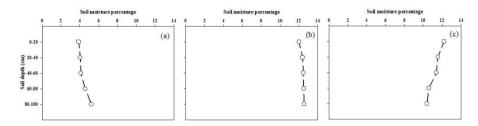


Figure 2. Soil moisture content (%) at 90 days after harvest (a), 180 days after harvest (b) and 270 days after harvest (c) with drought and recovery periods.

3.2. Germination and yield

In Thailand, milling season of sugar industry is from December to early May and it total milling period is about 5-6 months. Sugarcane harvested in the early milling season in December to January will encounter the drought period as the rainfed crop does not receive enough rain in the dry season. Drought can reduce ratooning ability of the crop and ultimately reduce sugarcane production. Breeding of sugarcane for high ratooning ability can help sugarcane growers to have higher income as the sugarcane can be harvested for more years before new planting.

In this study, germination of new plants from harvested plants of second ratoon crop was evaluated at 60 days after harvest (DAH), when the crop was subjected to drought period after harvest, and cane yield was evaluated at 270 DAH, when the crop received sufficient rain in the rainy season. The questions are that there are the variations in cane yield and germination percentage in these sugarcane genotypes and whether germination percentage is related to yield.

Sugarcane genotypes were significantly different ($P \le 0.01$) for cane yield and germination percentage compared to the first ration crop (Table 1). Cane yields ranging from 50.20 tons/ha in TBy28-0941 to 94.78 tons/ha in KKU99-02 were recorded among sugarcane genotypes, and germination percentages ranged from 35.19% in KK06-501 to 88.67% in UT13. The study indicated that wide variations among sugarcane genotypes were observed for cane yield and germination percentage. Germination percentage would be a valuable surrogate trait for cane yield of ration crops if the trait is associated with cane yield.

Table 1 Means for cane yield and germination percentage of the second ratoon crop of 17 sugarcane genotypes.

Genotype	Cane yield	Germination
Genotype	(tons/ha)	percentage (%)
KKU99-02	94.78a	$71.54^{ m abc}$
KK3	89.30 ^{ab}	81.91 ^{ab}

KK06-419	84.14 ^{abc}	55.56 ^{cd}
MPT02- 458	80.33a-d	63.73 ^{bc}
K88-92	78.89 ^{a-c}	70.46^{abc}
UT13	75.55a-f	88.67a
KKU99-01	$70.66^{\mathrm{a-g}}$	74.54abc
KKU99-06	67.59 ^{b-g}	75.58 ^{abc}
KKU99-03	$64.96^{\text{b-g}}$	79.41^{ab}
CSB07-219	63.47 ^{c-g}	38.16^{d}
CSB07-79	$58.66^{\mathrm{d-g}}$	76.56 ^{abc}
Kps01-12	56.11 ^{b-g}	63.47bc
TBy28- 1211	55.71 ^{efg}	70.29 ^{abc}
KK06-501	$55.39^{\rm efg}$	35.19 ^d
MPT05- 187	52.70 ^{fg}	60.60 ^{bc}
UT12	51.17^{fg}	68.99 ^{abc}
TBy28- 0941	50.2g	64.35 ^{bc}
Mean	67.63	67.00
F-test	**	**

^{**} significant at $p \le 0.01$. Mean in the same column followed by the same letters are not significant different at $P \le 0.05$ by LSD .

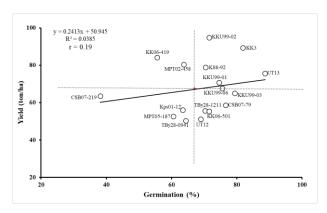


Figure 3 Relationship between germination percentage and cane yield of 17 sugarcane genotypes.

The sugarcane genotypes with high cane yield such as KKU99-02, KK3, K88-92, UT13 and KKU99-01 were associated with high germination percentage, whereas the sugarcane genotypes with low cane yield such as KK06-501 and CSB07-219 were associated with low germination percentage. The data also indicated that some sugarcane genotypes such as KK06-419 and MPT02-458 with high cane yield had intermediate germination percentage but the genotypes such as KK06-501 and CSB07-219 with extremely low germination percentage did not have high yield.

The relationships between germination percentage and cane yield is presented in Figure. 3. The correlation between germination percentage and cane yield was not significant. However, the relationship between these traits was positive although it was low (r=0.19). Germination of new plants from old plants is a critical event in sugarcane to assure good crop establishment and cane yield [12]. In this study the association of these traits was positive, indicating that germination percentage could contribute to cane yield for some extent. The associations of these traits was not strong could be possibly due to the effects of various factors that affect germination and cane yield.

Environmental factors greatly affect the ratooning ability and yield of ratoon crop [13,14]. The germinating bud is initially dependent on the nutrients and water in the set, and thereafter, it develops its own root system after about three weeks under proper conditions [15].

Soil water is also an important factor affecting germination of sugarcane. In Nigeria, the date of first irrigation after planting significantly affected sprouting percentage, and the best sprouting was obtained when sets were irrigated at planting [16]. Application superabsorbent polymer into the sandy soil to improve soil moisture content could increase germination and early growth of sugarcane [17].

In this study, irrigation was not available and the germination of the crop was dependent solely on stored soil water, and the authors evaluated the responses of sugarcane genotypes to the drought period at early growth phases of the second ratoon crop.

Sugarcane genotypes responded differently to soil moisture deficit for ratooning ability during drought period. UT13, KK3, KKU99-03, CSB07-79, KKU99-06, KKU99-01, KKU99-02, K88-92, TBy28-1211 and UT12 had high ratooning ability as indicated by high germination percentage in the second ratoon crop, whereas KKU99-02, KK3, K88-92, UT13 and KKU99-01 had high ratooning ability based on higher germination percentage and cane yield. Cane yield is a complex trait and, consequently, selection for can yield was generally done on the basis of yield components such as stalk number, stalk diameter, stalk length and single stalk weight. Effects of these traits on cane yield have been previously studied in the spring crop [18-20].

3.3. Root length density

Study of whole root systems under field conditions is difficult, and this might be possible only in greenhouse. Study of root length density using soil core method has been use to study roots of several crops under field conditions [21]. In this study, root length density was studied at two positions between rows and between plants in row and at 90 and 270 DAH. At the sampling times, the auger method was divided into five layers at 20 cm intervals and later the two upper layers were combined into one layer and three lower layer were combined into one layers because low recovery of the roots. The questions are that there are the variations in root length density in these sugarcane genotypes and which position of root length density is more informative.

Root length density was evaluated two times at 90 days after harvest (DAH) and 270 DAH at two positions between plants and between rows and at two soil depths at 0-40 cm and 40-100 cm (Table 2). Sugarcane genotypes were significantly different ($P \le 0.05$) for root length density at both soil depths, positions of sampling and sampling times. At 90 DAH, root length density between plants at 0-40 cm (1.16 cm) was higher than at 40-100 cm (0.24 cm). Similarly, at 270 DAH, root length density between plants at 0-40 cm (2.29 cm) was also higher than at 40-100 cm (0.28 cm). Root length densities between rows at 90 DAH were 0.18 cm for soil depth of 0-40 cm and 0.21 cm for soil depth of 40-100 cm, which were much lower than those between plants. Root length densities between rows at 270 DAH were 0.47 cm for soil depth of 0-40 cm and 0.21 cm for soil depth of 40-100 cm, which were lower than those between plants for root length densities at the soil depth of 0-40 cm and 40-100 cm.

At 90 days after harvest, root length densities between plants at soil depth of 0-40 cm ranged from 0.24 cm in KK06-419 to 1.73 cm in UT13, whereas root length densities at soil depth of 40-100 cm ranged from 0.10 cm in UT13 to 0.40 cm in KKU99-06. The sugarcane genotypes with high root length density at the soil depth of 0-40 cm were KK3, K88-92, UT13, KKU99-06, KKU99-03, Kps01-12, TBy28-1211, KK06-501 and TBy28-0941, whereas the sugarcane genotypes with high root length density at the soil depth of 40-100 cm were KKU99-06, Kps01-12 and UT12. KKU99-06 and Kps01-12 had high root length density at both soil layers of 0-40 and 40-100 cm.

Table 2 Root length density in 17 sugarcane genotypes on positioned between plants and between rows at the 90 and 270 days after harvest in upper soil layer (0 - 40 cm) and lower soil layer (40 - 100 cm).

Genotype	RLD (cm/cm³) between plants at 90 DAH	RLD (cm/cm³) between rowsat90DAH	RLD (cm/cm³) between plants at 270 DAH	RLD(cm/cm³) between rows at 270 DAH
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	Upper (040cm)	Lower (40-100 cm)	Upper (040 cm)	Lower (40-100 cm)	Upper (0-40 cm)	Lower (40-100 cm)	Upper (0-40 cm)	Lower (40-100 cm)
KKU99- 02	0.39 ^{de}	0.22 ^{d-g}	$0.27^{\rm cd}$	0.21 ^{def}	1.83 ^{d-g}	0.49^{a}	0.65 ^b	$0.27^{\rm cd}$
KK3	1.60^{a}	0.24^{def}	0.21^{de}	$0.23^{\rm cf}$	2.54 ^{be}	0.24 ^{eh}	0.27^{ef}	0.13^{gh}
KK06- 419	$0.24^{\rm e}$	0.28 ^{be}	0.36 ^b	0.23 ^{c-f}	3.49a	0.22 ^{e-h}	1.08a	0.42a
MPT02- 458	0.89bc	0.22 ^{efg}	0.06gh	0.25 ^{a-d}	1.95 ^{d-g}	$0.27^{\rm ef}$	0.21^{fg}	0,21 ^{def}
K88-92	1.46^{a}	0.19^{fg}	0.07gh	0.31ab	2.28 ^{def}	0.28def	0.29^{def}	0.30bc
UT13	1.73^a	0.10^{h}	0.58^a	$0.23^{\rm cf}$	1.59 ^{efg}	0.23e-h	0.63^{b}	0.14^{gh}
KKU99- 01	0.64^{cd}	0.22 ^{efg}	$0.27^{\rm cd}$	0.29 ^{abc}	1.84 ^{d-g}	0.27 ^{def}	0.12g	$0.17^{\rm efg}$
KKU99- 06	1.61ª	0.40^{a}	0.16^{ef}	0.19 ^{def}	3.43 ^{ab}	0.15 ^h	0.42 ^{cd}	0.28^{cd}
KKU99- 03	1.51ª	0.18^{fgh}	$0.15^{\rm ef}$	0.31 ^{ab}	2.48b-e	0.24 ^{e-h}	0.74^{b}	0.16 ^{eh}
CSB07- 219	0.58 ^{cde}	0.16gh	0.13 ^{efg}	0.10gh	$2.70^{\mathrm{a-d}}$	0.26 ^{efg}	0.12 ^g	0.10gh
CSB07-79	0.89bc	0.29 ^{bcd}	0.01^{h}	$0.18^{\rm ef}$	1.35^{fg}	0.32^{cde}	0.21^{fg}	0.29bc
Kps01-12	1.59a	0.35ab	0.29bc	0.32^{a}	1.16^{g}	0.21^{fgh}	0.74^{b}	0.22^{de}
TBy28- 1211	1.55a	0.26 ^{cf}	0.07gh	0.10gh	2.39 ^{cde}	0.37 ^{bcd}	0.10g	0.15eh
KK06- 501	1.53a	0.25 ^{cf}	0.09 ^{fgh}	0.17^{fg}	2.33 ^{cde}	0.38bc	0.47 ^c	0.15 ^{fgh}
MPT05- 187	1.00b	$0.21^{\rm efg}$	0.26 ^{cd}	0.20 ^{def}	2.70 ^{a-d}	0.47 ^{ab}	0.36 ^{cde}	0.35 ^b
UT12	1.03b	0.32 ^{abc}	0.05^{h}	0.24 ^{b-e}	$1.72^{\rm efg}$	0.16^{gh}	1.10^{a}	0.09h
TBy28- 0941	1.54ª	0.11 ^h	0.09 ^{fgh}	0.07h	3,24 ^{abc}	0.18 ^{fgh}	0.44°	0.16eh
Mean F-test	1.16 **	0.24	0.18 **	0.21 **	2.29	0.28	0.47 **	0.21 **

^{**} significant at $p \le 0.01$. Mean in the same column followed by the same letters are not significant different at $P \le 0.05$ by LSD .

At 90 days after harvest, root length densities between rows at the soil layer of 0-40 cm ranged from 0.01 cm in CSB07-79 to 0.58 cm in UT13, whereas root length densities at the soil layer of 40-100 cm ranged from 0.07 cm in TBy28-0941 to 0.32 cm in Kps01-12. UT13 had high root length density at the soil layer of 0-40 cm, and MPT02-458, KKU99-03 and Kps01-12 had high root length density at soil layer of 40-100 cm. No sugarcane genotype showed high root length density at both soil layers.

At 270 days after harvest, root length densities between plants at the soil layer of 0-40 cm ranged from 1.16 cm in Kps01-12 to 3.49 cm in KK06-419, whereas root length densities at the soil layer of 40-100 cm ranged from 0.15 cm in KKU99-06 to 0.49 cm in KKU99-02. The sugarcane genotypes with high root length density at soil layer of 0-40 cm were KK06-419, KKU99-06, CSB07-219, MPT05-187 and TBy28-0941, whereas the sugarcane genotypes with high root length density at soil layer of 40-1400 cm were and KK06-419 and MPT05-187. MPT05-187 had high root length density at both soil layers of 0-40 and 40-100 cm.

At 270 days after harvest, root length densities between rows at the soil layer of 0-40 cm ranged from 0.10 cm in TBy28-1211 to 4.10 cm in UT12, whereas root length density at the soil layer of 40-100 cm ranged from 0.09 cm in UT12 to 0.42 cm in KK06-419. The sugarcane genotypes with high root length density at soil layer of 0-40 cm were KK06-419 and UT12. The sugarcane genotype (KK06-419) with high root length density at soil layer of 0-40 cm also In this study, variations in root length

density were found in upper soil layers and lower soil layers at two positions and at 90 and 270 DAH. Root length density at the position between rows was much lower than the position between plants with in rows possibly due to the plants in the rows were much closer than the plants between rows. However, root length density at the position between rows was still useful.

Roots provide anchorage and facilitate acquisition of water and nutrients from the soil [22]. Sugarcane is a deep rooted crop owing to its long growth cycle and longevity of the root system through multiple rotations compared to other crops. Root system reaches to a depth of 1.5 meters and even 6 meters [4]. However, drought conditions had been reported to increase root system for seeking over water from the inadequate soil moisture environment sources [23].

In this study, sugarcane genotypes under drought stress condition had high root length density in lower soil layers at the position between plants and at the position between rows. KK3, K88-92 and UT13 showed high root length density in upper soil layers at the position between plants. UT13 had high in upper soil layer at the position between rows, whereas K88-92 and KKU99-01 showed high RLD in lower soil layers.

Deep rooting is a complex trait consisting of root growth angle and maximum root length [24,25]. Phenotypic variation in the root growth angle and root length density at the position between plants has been found to steep and near-vertical root angle [26]. Moreover, root length density at the position between rows has been found to near-horizontal root angle. Root growth angle determines whether a plant develops a shallow or deep root system.

3.4. Root distribution patterns

Drought may modify root growth of sugarcane and sugarcane genotypes may respond differently for root growth. The sugarcane genotypes were classified into three main groups for each evaluation time at 90 DAH and 270 DAH based on root distribution patterns of root length density at two positions between rows and between plants within row (Figure 4, 5). Each group has two subgroups, therefore, there were six main groups and 12 sub-groups altogether.

At 90 DAH, ten sugarcane genotypes consisting of TBy28-1211, K88-92, KKU99-03, TBy28-0941, KK06-501, UT12, KKU99-06, KK3, Kps01-12 and UT13 were classified into group 1, which was characterized by high root length density in the upper soil layers and low root length density in the lower soil layers at the position between plants (Figure 4). The sub-group 1.1 had seven sugarcane genotypes consisting of TBy28-1211, K88-92, KKU99-03, TBy28-0941, KK06-501, UT12, and KKU99-06, whereas the sub-group 1.2 had three sugarcane genotypes including KK3, Kps01-12 and UT13. The sub-group 1.1 had low root length density in the upper soil layers and high root length density in the lower soil layers at the position between rows, whereas the sub-group 1.2 had high root length density in the upper soil layers at the position between rows.

Group 2 had four sugarcane genotypes consisting of CSB07-79, CSB07-219, MPT02-458 and KKU99-01. The genotypes in this group had high root length density in the upper soil layers and in the lower soil layers at the position between plants. CSB07-79, CSB07-219 and MPT02-458 formed the groups 2.1 as they had low root length density in the upper soil layers and high root length density in the lower soil layers at the position between rows, and KKU99-01 was classified into the group 2.2 as they had high root length density in the upper soil layers and high root length density in the lower soil layers at the position between rows.

Group 3 had three sugarcane genotypes consisting of MPT05-187, KK06-419 and KKU99-02. The sugarcane genotypes in this group had high root length density in the upper soil layers and in the lower soil layers at the position between rows. KK06-419 and KKU99-02 were classified into subgroup 3.1 because its low root length density in the upper soil layers and high root length density in the lower soil layers at the position between plants, whereas MPT05-187 had low root length density in the upper soil layers and in the lower soil layers at the position between plants.

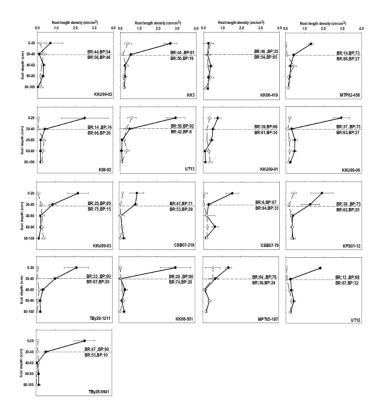


Figure 4 Root length density of 17 sugarcane genotypes in five soil layers at 20 cm intervals (0-40 cm for upper soil layers and 40-100 cm for lower soil layers) evaluated at two positions between plant (BP) () and between row (BR) () at the 90 days after harvest.

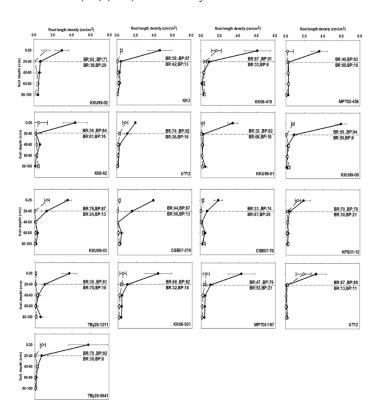


Figure 5 Root length density of 17 sugarcane genotypes in five soil layers at 20 cm intervals (0-40 cm for upper soil layers and 40-100 cm for lower soil layers) evaluated at two positions between plant (BP) (9 and between row (BR) () at the 270 days after harvest.

At 270 DAH, eight sugarcane genotypes consisting of TBy28-0941, KK06-501, MPT05-187, K88-92, CSB07-219, KK3, KKU99-06 and KK06-419 were classified into group 1 (Figure 5). The sub-group 1.1 had six sugarcane genotypes consisting of TBy28-0941, KK06-501, MPT05-187, K88-92, CSB07-219 and KK3, whereas the sub-group 1.2 had two sugarcane genotypes including KKU99-06 and KK06-419. The sub-group 1.1 had high root length density in the upper soil layers and low root length density in the lower soil layers at the position between plants, whereas, had low root length density in the upper soil layers and high root length density in the lower soil layers and high root length density in the upper soil layers and high root length density in the upper soil layers and high root length density in the lower soil layers and high root length density in the lower soil layers at the position between plants and position between rows.

Group 2 had six sugarcane genotypes consisting of TBy28-1211, MPT02-458, KKU99-01, UT12, KKU99-02 and KKU99-03. The genotypes in this group had high root length density in the upper soil layers and low root length density in the lower soil layers at the position between plants. TBy28-1211, MPT02-458 and KKU99-01 formed the groups 2.1 as they had low root length density in the upper soil layers and lower soil layers at the position between rows. UT12, KKU99-02 and KKU99-03 were classified into the group 2.2 as they had high root length density in the upper soil layers and low root length density in the lower soil layers at the position between rows.

Group 3 had three sugarcane genotypes consisting of UT13, CSB07-79 and Kps01-12. The sugarcane genotypes in this group had high root length density in the upper soil layers and low root length density in the lower soil layers at the position between rows. UT13 and CSB07-79 were classified into sub-group 3.1 because its low root length density in the upper soil layers and high root length density in the lower soil layers at the position between plants, whereas Kps01-12 had low root length density in the upper soil layers and in the lower soil layers at the position between plants.

In this study, the well-watered control was not available. Under well-water conditions, root density was high in the upper soil layers. However, under drought conditions, plants increased root systems to take up more water from the inadequate soil moisture environments [23]. According to [27] drought could modify root growth to water uptake to meet the demand of the plant.

In this study, sugarcane genotypes were significantly different for germination percentage. Germination and development of sugarcane were closely related to soil moisture [28]. KKU99-06 showed high root length density at both soil layers of 0-40 and 40-100 cm under drought (at 90 DAH). The results indicated high root length density and good root distribution under drought supported germination.

Drought limits growth and physiological processes and, therefore, it reduces yield of sugarcane [7,29] and plant height is most severely affected by drought [30]. Yield loss by drought would be as high as 60 percent [1].

Root is an important plant part that takes up nutrients and water to shoot and it is directly related to yield. Sugarcane genotypes might have drought tolerant mechanisms to cope with a water limited environments by maintaining high water status and investing more assimilate proportion for supplying root system during drought stress. The maintenance of high water uptake under drought stress might be assisted by improvement of root/shoot ratio, and the drought resistant cultivars are able to maintain high root/shoot ratio under drought [31].

3.5. Physiological traits related to drought resistance

SPAD chlorophyll meter reading (SCMR), chlorophyll fluorescence and relative water content were evaluated at 90, 180 and 270 DAH, whereas specific leaf area and stomatal conductance were evaluated at 180 and 270 DAH. Sugarcane genotypes were significantly different (P≤0.05) for SCMR at 180 and 270 DAH, chlorophyll fluorescence at 90 and 180 DAH and relative water content at 90 DAH (Table 3). KKU99-02, CSB07-79, TBy28-1211 and MPT05-187 had the highest SCMR at 180 DAH, and TBy28-1211 also had the highest SCMR at 270 DAH. TBy28-1211 and UT12 had the highest chlorophyll fluorescence at 90 DAH, whereas KK06-419, MPT02-458, K88-92, UT13, KKU99-01,

KKU99-06, TBy28-1211, Kps01-12, TBy28-1211 and MPT05-187 had the highest chlorophyll fluorescence at 270 DAH.

Relative water content at 90 DAH (96.20%) was lower than at 180 and 270 DAH (98.26 and 96.61, respectively). Although sugarcane genotypes were significantly different for relative water content at 90 DAH, the differences were rather small and most sugarcane genotypes were similar except for MPT02-458, Kps01-12 and KK06-501, which had the lowest relative water content.

Sugarcane genotypes were significantly different (≤P0.05) for specific leaf area at 180 and 270 DAH and stomatal conductance at 180 and 270 DAH (Table 4). Specific leaf area at 180 DAH (82.99 cm²/g) was higher than at 270 DAH (66.47 cm²/g), whereas stomatal conductance at 180 DAH (304.32 µm²/s) was lower than at 270 DAT (338.20 µm²/s). The genotypes with low specific leaf area are preferable as they show thicker leaves. KK3, K88-92, CSB07-219, CSB07-219, KK06-501, MPT05-187 and UT12 had the lowest specific leaf area at 180 DAH, KK3, KK06-419, MPT02-458, K88-92, Kps01-12, UT12 and TBy28-0941 had the lowest specific leaf area at 270 DAH. KK3, K88-92 and K88-92 had consistently high specific leaf area across sampling dates.

The sugarcane genotypes with high values are preferable. KK3, KK06-419, MPT02-458, UT13, KKU99-01, KKU99-06, KKU99-03, Kps01-12 and TBy28-1211 had the highest stomatal conductance at 180 DAH, and KK06-419, Kps01-12, TBy28-1211 and MPT05-187 had high specific leaf area at 270 DAH, KK06-419, Kps01-12 and TBy28-1211 showed high stomatal conductance at both 180 DAH and 270 DAH.

Table 3 SPAD chlorophyll meter reading (SCMR), Chlorophyll fluorescence and Relative water content (RWC) of the 2nd ratoon cane in 17 sugarcane genotypes.

	` '			Ŭ	0 71				
		SCMR		- Chloro	phyll fluor (Fv/Fm)	rescence	_	RWC (%)
Genotype	90	180	270		180	270		180	
	DAH	DAH	DAH	90DAH	DAH	DAH	90DAH	DAH	270DAH
KKU99-02	38.51	43.97a	40.21 ^{bcd}	0.772^{ef}	0.782^{e}	0.816	96.00abc	98.44	92.39
KK3	36.06	40.90bcd	39.66 ^{cd}	0.803ae	0.799 ^{cde}	0.803	94.98a-d	99.24	96.65
KK06-419	37.46	40.99 ^{bcd}	39.59 ^{cd}	0.799a-f	0.813 ^{abc}	0.816	97.58ab	98.80	99.11
MPT02- 458	36.53	40.34 ^{cd}	39.89 ^{cd}	0.781 ^{cf}	0.811 ^{abc}	0.806	93.93 ^{bcd}	98.13	98.49
K88-92	39.12	$40.17^{\rm cd}$	40.09cd	0.770 [£]	0807a-d	0.828	97.59ab	99.32	97.56
UT13	36.84	$40.17^{\rm cd}$	41.27^{bcd}	0.778^{def}	0.813 ^{abc}	0.822	98.29a	97.90	98.83
KKU99-01	37.97	40.92 ^{bcd}	40.32 ^{bcd}	0.810abc	$0.808^{\mathrm{a-d}}$	0.813	97.36 ^{abc}	97.88	98.41
KKU99-06	38.59	38.66^{d}	41.24^{bcd}	0.794b-f	0.804a-d	0.812	95.36 ^{a-d}	99.13	98.60
KKU99-03	37.98	40.10^{d}	42.29bc	0.789^{b-f}	0.797^{cde}	0.799	95.73 ^{a-d}	97.71	96.90
CSB07-219	35.56	$40.32^{\rm cd}$	40.32^{bcd}	0.819^{ab}	$0.805^{\mathrm{a-d}}$	0.804	95.59 ^{a-d}	99.26	97.71
TBy28- 1211	35.78	42.31 ^{abc}	40.60 ^{bcd}	0.828a	0.820a	0.805	96.84abc	97.94	97.61
Kps01-12	36.87	38.95^{d}	$39.64^{\rm cd}$	$0.768^{\rm f}$	0.807^{a-d}	0.813	93.66 ^{cd}	98.28	90.90
TBy28- 1211	39.61	44.93a	46.00a	0.776 ^{def}	0.818ab	0.821	98.69a	97.13	99.01
KK06-501	37.06	41.09 ^{bcd}	42.93 ^b	0.777^{de}	0.792^{de}	0.803	92.24 ^d	98.96	95.75
MPT05- 187	39.99	43.59ab	41.65 ^{bcd}	0.780 ^{c-f}	0.810 ^{abc}	0.797	98.27ª	95.97	98.50
UT12	35.51	40.3 ^{cd}	38.94^{d}	0.807a-d	0.790^{de}	0.797	97.89a	97.32	98.46
TBy28- 0941	35.51	40.26 ^{cd}	41.60 ^{bcd}	0.775ef	0.799 ^{b-e}	0.801	95.38 ^{a-d}	99.14	97.48
Mean	37.43	41.06	40.95	0.789	0.805	0.808	96.20	98.26	96.61
F-test	ns	**	***	**	*	ns	*	ns	ns

ns, *, ** non-significant and significant at $p \le 0.05$ and $p \le 0.01$, respectively. Mean in the same column followed by the same letters are not significant different at $P \le 0.05$ by LSD .

genetypeer				
Genotype	Specific leaf	area (cm²/g)	Stomatal cond	uctance (µm²/s)
	180 DAH	270 DAH	180 DAH	270 DAH
KKU99-02	83.14^{def}	66.58 ^{b-e}	$264.87^{\rm ef}$	354.78 ^{bcd}
KK3	62.99 ^h	57.58^{ef}	358.05a	316.20^{def}
KK06-419	91.30bcd	55.37 ^f	330.10ab	372.90^{ab}
MPT02-458	98.12 ^{bc}	58.34^{ef}	318.73 ^{abc}	317.95^{def}
K88-92	68.36 ^h	54.97^{f}	$258.33^{\rm ef}$	$272.48^{\rm g}$
UT13	88.75 ^{b-e}	69.13 ^{bcd}	317.15^{a-d}	326.33^{c-f}
KKU99-01	85.60c-f	74.46^{b}	336.68ab	356.48 ^{bcd}
KKU99-06	101.29ь	69.46 ^{bcd}	353.28a	324.13^{c-f}
KKU99-03	119.76a	71.45^{bc}	322.07 ^{ab}	327.38^{c-f}
CSB07-219	65.08h	68.75 ^{bcd}	251.40^{f}	355.33 ^{bcd}
CSB07-219	65.13 ^h	67.41 ^{b-e}	$269.68^{\rm ef}$	342.68^{b-e}
Kps01-12	84.95c-f	61.11^{def}	353.47a	380.00^{ab}
TBy28-1211	82.57^{d-g}	71.27^{bc}	343.20ab	365.75 ^{abc}
KK06-501	75.58 ^{e-h}	72.52 ^b	$246.00^{\rm f}$	337.10 ^{b-e}
MPT05-187	73.87^{fgh}	84.96a	272.83 ^{def}	408.28^{a}
K88-92	68.88gh	64.53^{b-f}	275.15 ^{c-f}	$291.03^{\rm fg}$
TBy28-0941	95.55 ^{bcd}	61.96c-f	302.50^{b-e}	$300.68^{\rm efg}$
Mean	82.99	66.47	304.32	338.20
F-test	**	**	**	**

Table 4 Specific leaf area (SLA) and stomatal conductance of the second ration cane in 17 sugarcane genotypes.

In general, drought reduces soil water potential, leaf water potential [32,33] and photosynthetic efficiency of photosystem II [7]. Drought also reduces relative water content [7,34] stomata conductance [35] photosynthetic rate [36-38] and PSII photochemical efficiency [35].

In this study, KK3, KK06-419 and KKU99-01 increased chlorophyll fluorescence and relative water content under drought stress period. They might have drought tolerance mechanisms to maintain high water status under drought. Maintenance of high water status was possibly due to the ability of root to mine more water under drought [39].

In this study, sugarcane genotypes were not significantly different for SCMR at 90 DAH. In previous study, early season drought significantly reduced SCMR in sugarcane [40]. According to [41] SCMR is an indicator of the photo-synthetically active light-transmittance characteristics of leaf, which is dependent on the unit number of chlorophyll per unit leaf area (chlorophyll density). SCMR was also closely related to chlorophyll content [42] and chlorophyll density [43]. The differences in the results among different studies might be due to the differences in plant ages, plant genotypes and drought duration and severity.

KK3 and K88-92 had low specific leaf area at 180 DAH, which was the period that the crop was recovered from drought, indicating that this genotype had thicker leaves. Thicker leaves usually have higher chlorophyll per unit leaf area and hence had a greater photosynthetic capacity.

KK06-419 showed high stomatal conductance after recovery period. Stomatal conductance of sugarcane had been controlled by roots, and might also involve in chemical signal [44]. In sugarcane, drought-tolerant genotypes could maintain high relative water content under drought [45]. KKU99-02, KK3, KK06-419, K88-92, UT13 and KKU99-01 had high relative water content under drought. In sugarcane, the cultivars with drought tolerance had higher relative water content and stomatal conductance than did the susceptible cultivars [46].

Enhanced root length in the lower soil layers enables plants to better extract available soil moisture from soil profile [23,47]. Root system of plant responds to soil water both water limited soil and water abundant soil. Root growth deep into the lower soil results in the increase in relative water

^{**} significant at p \leq 0.01. Mean in the same column followed by the same letters are not significant different at P \leq 0.05 by LSD .

content of about 40–51% [48]. [49] reported that roots and physiological responses such as root length, chlorophyll fluorescence and stomatal conductance at stressed period were important factors contributing to sugarcane biomass yield.

3.6. Correlations among traits

The correlation coefficients of root length density with cane yield and germination percentage were presented in Table 5. Most correlation coefficients were not statistically significant. However, significant correlation coefficients were observed for root length density between plants in upper soil layers at 90 DAH and germination percentage (0.36**), root length density between plants in lower soil layers at 270 DAH and germination percentage (-0.32*), root length density between rows in lower soil layers at 90 DAH and cane yield (0.35*) and root length density between rows in lower soil layers at 270 DAH and cane yield (0.51**).

Root length density between plants at early growth phases (90 DAH) in the upper soil layers might be beneficial to germination percentage, whereas germinated plants may reduce root length density between plants at 270 DAH in the lower soil layers. In contrast, germinated plants increased root length density between rows at 90 and 270 DAH in the lower soil layers. The negative correlation of root length density between plants in lower soil layers at 270 DAH and germination percentage might be due to competition for assimilates between shoots and roots. Where water is available in the surface layers of the soil following harvest, new shoots are sustained by new superficial roots, but that persistence of the old root system is critical to survival of the stool and growth of the ratoon crop during dry periods after harvest [50].

Table 5 Correlation coefficient (r) of root length density (RLD) on cane yield and germination percentage of the 2nd ratoon cane of 17 sugarcane genotypes.

	Cane yield ¹	Germination percentage ¹
Root length density		
Upper soil layer (0 – 40 cm)		
RLD between plants at 90 DAH	-0.12	0.36**
RLD between rows at 90 DAH	0.22	0.14
RLD between plants at 270 DAH	0.17	-0.14
RLD between rows at 270 DAH	-0.09	-0.01
Lower soil layer (40 - 100 cm)		
RLD between plants at 90 DAH	0.12	-0.00
RLD between rows at 90 DAH	0.35*	0.12
RLD between plants at 270 DAH	-0.00	-0.32*
RLD between rows at 270 DAH	0.51**	-0.12

^{*, **} significant at 0.05 and 0.01 probability level, respectively. ¹The data were calculated from treatment means with degree of freedom of 67.

Table 6 Correlation coefficients (r) of physiological traits (SPAD chlorophyll meter reading (SCMR), Chlorophyll fluorescence and Relative water content (RWC), specific leaf area (SLA) and stomatal conductance) on cane yield and germination percentage of the 2nd ration cane of 17 sugarcane genotypes.

	Cane yield ¹	Germination percentage ¹
Physiological traits		
At 90 DAH.		
Chlorophyll fluorescence	-0.01	0.05
RWC	0.13	0.27*
SCMR	0.21	0.21
At 180 DAH.		

Chlorophyll fluorescence	0.44**	0.27
RWC	0.06	-0.07
SCMR	0.04	-0.23
SLA	0.18	0.14
Stomatal conductance	0.36**	0.45**
At 270 DAH.		
Chlorophyll fluorescence	0.19	-0.03
RWC	0.10	0.21
SCMR	-0.07	0.01
SLA	-0.26	-0.05
Stomatal conductance	0.08	-0.16

^{*, **} significant at 0.05 and 0.01 probability level, respectively. ¹The data were calculated from treatment means with degree of freedom of 67.

It is interesting to note here that root length density between rows in lower soil layers at 90 and 270 DAH supported can yield of sugarcane. These root distributions might be due to adaptation to drought and had benefits to sugarcane to take up more water in the lower soil layers. Row distance is an important factor affecting root distribution patterns of sugarcane [51]. Wide distance of rows may provide more soil moisture to the crop during water limited conditions.

Physiological traits that had significant correlations with germination percentage were relative water content at 90 DAH (0.27*) and stomatal conductance at 180 DAH, whereas physiological traits what had significant correlations with cane yield were chlorophyll fluorescence at 180 DAH (0.44**) and stomatal conductance at 180 DAH (0.36**) (Table 6). Physiological traits at 270 DAH were not correlated with cane yield and germination percentage.

Soil moisture is important factor for germination of sugarcane as indicated by high correlation of germination and relative water content at 90 DAH. Germinated plants might then increase stomatal conductance and ultimately increase can yield. Stomatal and root hydraulic conductances are correlated in sugarcane, for both pot and field-grown crops, resulting in approximately homeostatic regulation of leaf water potential [4].

Chlorophyll fluorescence at 90 DAH is also another trait that was related to yield at harvest, whereas all physiological traits at 270 DAH were not related to germination percentage and cane yield. The significant correlation between chlorophyll fluorescence and cane yield is not surprising because this trait is related to photosynthesis [52]. Chlorophyll fluorescence might be useful as a surrogate trait for cane yield. Evaluation of physiological traits at 270 DAH may be too late to find the significant relationships on cane yield.

4. Conclusion

The sugarcane genotypes were significantly different for germination percentage and cane yield, but the correlation between these traits were low although it was positive. The results indicated that reduction in germination percentage could lead to lower can yield. Selection of sugarcane genotypes with medium to high germination percentage should improve ratooning ability in these sugarcane population. The correlation between root length density and germination percentage indicated that root length density between plants at 90 DAH in the upper soil layers might contribute to high germination percentage and root length density between rows at 90 and 270 DAH in the lower soil layer might contribute to high cane yield.

Relative water content at 90 DAH was significantly correlated with germination percentage, indicating that high water uptake of plants is important for high germination. Chlorophyll fluorescence and stomatal conductance were positively associated with germination percentage and cane yield, indicating that these traits contribute to cane yield for some extent.

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