INTRODUCTION

Sugarcane is a major economic crop in many countries (Sanghera, Malhotra, Singh, & Bhatt, 2019), such as Brazil, Australia and Thailand. It occupied an area of 28.4 million ha in 2016, with a total production of 201.3 million tons and an average fresh cane yield of 5,868.61 t/ha (FAO, 2018). Sugarcane is mainly produced under rain-fed system, in the semi-arid regions. In these conditions, water shortage is a main production constraint, as precipitation is mostly unpredictable and inadequate (ETWWA, 2010). In Thailand, sugarcane cultivation is usually exposed to drought stress around 1–4 months after planting. Water-deficit during the early growth stage significantly disturbs cane growth and yield (Zhao & Li, 2015; Zhao, Glaz, & Comstock, 2010). Growing sugarcane cultivars that are tolerant to these drought conditions is a strategy for the mitigation of drought. Thus, an understanding of mechanisms to overcome drought in these plants is required.

Elite sugarcane clones that can maintain root and shoot dry matter accumulation to a satisfactory level under drought stress conditions are preferred. Dehydration avoidance of plant includes mechanisms to reduce transpiration by maintaining high water status and water uptake during stress condition (Kooyers, 2015). Drought resistance mechanisms in relation to the above-ground portion of the plant have been discussed in several reports by Jangpromma, Thammasirirak, Jaisil, & Songsri (2012) and Zhao & Li (2015). Transpiration rate, stomatal conductance, leaf and canopy temperature, canopy conductance, photosynthesis rate and leaf chlorophyll content (SPAD index) are reported as indirect selection criteria for drought resistance of sugarcane cultivar (Basnayake, Jackson, Inman-Bamber, & Lakshmanan, 2015; da Silva et al., 2012; Endres, Silva, Ferreira, & De Souza Barbosa, 2010). Furthermore, a positive relationship between root and shoot growth has been identified (Smith, Lawn, & Nable, 1999). As the prior organ to sense moisture
inadequate in the soil and to signal the constraint to other structure is the root, an understanding of the roots traits that can tolerate stress will help to improve crop production via provide more perceptions of research strategies (Ferreira et al., 2017). Root is a key trait to support shoot development of sugarcane (Smith, Inman-Bamber, & Thorburn, 2005). Nutrient and water are taken up by the roots, to support physiological processes (Henry, 2013; Mathieu, Lobet, Tocquin, & Périlleux, 2015). The root is also involved with transpiration, which is related to the stomatal conductance (Smith, Lawn, & Nable, 1999). Consequently, it plays a major role in the drought resistance (Henry, 2013). Sugarcane roots are divided into two groups, sett and shoot roots. The initial root system is the sett root, while shoot root accounts for the main root system in approximately 3 months after planting (Smith, Inman-Bamber, & Thorburn, 2005). A high root dry weight, with longer root length, increased root surface area and root volume are the preferred traits for drought tolerance since they can extract more water under water-shortage conditions (Jangpromma, Thammasirirak, Jaisil, & Songsri, 2012; Khonghintaisong, Songsri, Toomsan, & Jongrungklang, 2018). Nevertheless, root studies under normal field conditions are difficult and complex, because it relies on separating the root from the soil by the washing process. Root sample may be lost in the process, leading to an error in the result interpretations. Moreover, sample collection in the field consumes considerable time and labour (Girdthai et al., 2010). Therefore, a novel method that can circumvent all these complex steps is necessary.

The soil-less methodology is a technology for plant production that can control environmental factors and diseases (Trejo-Téllez & Gómez-Merino, 2012; Wahome, Oseni, Masarirambi, & Shongwe, 2011). A hydroponic system is an apt one for conducting the research (Alatorre-Cobos et al., 2014), so it may be useful in root investigation, simplifying the washing process. In Arabidopsis, a hydroponic system provides many advantages for root research, such as root environmental control, and complete and easy access to the root samples (Mathieu, Lobet, Tocquin, & Périlleux, 2015). The root growth of tomato grown under hydroponic system and exposed to electric field was studied by Tataranni, Sofo, Casucci, & Scopa (2013).

To date, there is limited information available on the relationship among root and shoot traits for sugarcane grown under hydroponic conditions. An earlier work revealed a negative correlation between the root traits and panicle of wheat in response to drought stress, artificially imposed by polyethylene glycol-treated hydroponic culture (Robin, Uddin, & Bayazid, 2015).

However, there has been no prior investigation into sugarcane grown under hydroponic conditions. Hence, the aim of this research was to investigate the root properties, including dry weight, root length, root surface area and root volume of diverse sugarcane cultivars grown under hydroponic conditions and their relationship with the shoot growth, along with the correlation of root traits from hydroponic studies with that from field studies. This information would be useful in developing an alternative method for investigating drought resistance with the help of root studies and associated measurements. The information might also considered reliable as those field data field data in plant breeding, and also for investigating nutrient use efficiency of diverse sugarcane genotypes.

**MATERIALS AND METHODS**

**Hydroponics Experiment**

**Plant Materials and Experimental Design**

The hydroponic trial was conducted under open greenhouse conditions at the Field Crops Research Station of Agronomy Department, Khon Kaen University, Thailand (latitude 16° 28’ N, longitude 102° 48´ E, 200 masl) from February to May 2016. Aeration system was constructed by Dynamic Root Floating Technique (DRFT) (Ortiz, Rotatori, Schreiber, & von Roth, 2009). The experiment was designed in RCBD with four replications. Eight sugarcane genotypes with different drought resistance levels, i.e CSB06-2-15, CSB06-4-162, CSB06-5-20, NSUT08-22-3-13, KPS01-12, TBy07-1385, KK07-478 and KK3, were used in the study. CSB06-2-15, CSB06-4-162, CSB06-5-20 and NSUT08-22-3-13 were considered as susceptible whereas KPS01-12 and KK3 were categorized as drought-resistant cultivars (OCSB, 2014). KK07-478 and TBy07-1385 were the elite cultivars in Thailand’s sugarcane breeding program and have not been studied for their drought resistance. The experiment was then served as the evaluation for these elite lines for their response to drought resistancy.
Experimental Management

Ten months after planting, cane stalks were cut into single budded setts. Soil and filter cake were mixed at 1:1 ratio (v/v), to fill a plastic bag. Single bud setts were planted and irrigated immediately for obtaining uniform germination. Cane settlings were transplanted to the hydroponic system at 10 days after planting. Each hydroponic block was 70 cm wide × 320 cm length, with a depth of 30 cm. A block contained eight cane settlings, arranged in two rows, with four plants per row (Fig. 1). The distance between the planted seedlings was arranged 30 × 80 cm. The hydroponic system was filled with water at a pH of 7.05 with an electrical conductivity (EC) of 0.8 ds/m. Liquid fertilizers namely A and B were mixed with water at the concentration of 1:100 (v/v) and applied monthly. At 50 l, fertilizer solution of A contained Ca(NO₃)₂ 5.5 kg, Fe-EDTA 80 g while fertilizer solution B was constituted of NH₄H₂PO₄ 435 g, KNO₃ 5 kg, MgSO₄ 2.82 kg, KPO₄ 875 g, Cu-EDTA 2 g, Zn-EDTA 5.5 g and Mn-EDTA 9 g. Disease and insect were controlled as needed, to keep the canes free from pests and diseases throughout the experiment.

Data Collections

The data of daily maximum and minimum temperature, relative humidity (RH), evaporation (E₀), etc., were collected, from the time of transplanting till the end of the study (around 3 months after transplanting) by a meteorological station placed at a distance of 20 m from the studied area. This work was operated from February to May 2016. The maximum air temperature range was 28.5–43.0 °C and the minimum temperature ranged from 14.5 –30.0 °C during the experimental period. Both air temperatures had a similar pattern, with low values during the early investigation period that then increased during the middle and terminal periods (Fig. 2). The E₀ (range, 3.8–10.9 mm) and RH (range, 56–97 %) revealed normal conditions occurred during this experimental period (Fig. 2). Therefore, meteorological conditions should not have disturbed the normal growth of sugarcane, in this study.

Root samples were collected without sub-sampling, and from all four replications. Root volume root length, and root surface area were measured at 3 months after transplanting. Root samples of the plant were washed at flowing water to eliminate contaminants from the roots. Winrhizo software program (Winrhizo Pro (s) V. 2004a, Regent Instruments, Inc.) was used for the measurement of root length, root volume and root surface area. After drying under oven at 80 °C for 48 h, dry weight of root samples were observed.

Fig. 1. Seedlings of the evaluated sugarcane cultivars after 30 DAP grown under the hydroponic system
Shoot samples, including stem and leaves were collected on the same date as the root measurements. Stem height was measured from the base up to top of primary tiller, while the stem diameter was observed from the middle part of stem using caliper. The stem and leaf samples were dried by oven under similar temperature range and period with that of the root samples, and the dry weights were then recorded.

Field Experiment
Field experiment was conducted at the farmers' field, Udon Thani, Thailand (latitude 17° 10’ N, longitude 102° 47’ 43’E, 187 m asl) from December 2015 to March 2016. The soil type of the experimental area was loamy sand and an RCBD experiment with three replication was established to further evaluate the eight sugarcane genotypes.

Sugarcane setts with three buds were at the previously prepared plots. Each plot was arranged in 60 m² with 7.5 m wide and 8 m long. The distance between row was 150 cm and between plants was 50 cm. Plot size was 60 m² with 7.5 m in width and 8 m in length and spacing of 150 cm between rows and 50 cm between plants. The field test was carried out under rain-fed conditions. Initial 50 kg N/ha, 50 kg P/ha and 25 kg K/ha were applied immediately as basal fertilizers after planting. After 3 months planting, supplemental fertilizers constituted of 175 kg N/ha, 58 kg P/ha and 150 kg K/ha was applied. Weed control was performed as needed to keep the canes free from weeds during the experiment.

Data Collections
Minimum and maximum temperature, RH, $E_o$ were noted daily, from the time of planting until the last data collection (around 3 months after planting) from the weather station situated at a distance of 39.9 km from the experiment. This field trial was conducted during December 2015 to March 2016. The maximum air temperature range was recorded 19.4–41.5 °C and the minimum temperature ranged from 7.8 –24.8 °C, during the experimental period. The $E_o$ (range, 0.29–8.18 mm) and RH (range, 41.13–83.13 %) revealed normal conditions occurred during this experimental period.

For field test, root samples were collected at 4 months after planting using the auger method (Jongrungklang et al., 2011). The size of the coring tube was 1.15 m in length and 76 mm in a diameter. Root samples were taken from a depth of 100 cm. In the root washing process, the round sieves with mesh size of 0.5 mm² as recommended by Jongrungklang et al. (2011) was used. Root samples were washed manually to clean up the samples from the soil and debris. Root length was measured with the WinRHIZO program. Root length density was determined as a calculation of the ratio between root length (cm) and soil volume (cm³).
Statistical Analysis

Analysis of variance (ANOVA) of each trial was conducted using statistical software of Statistix ver. 8 (copyright 1985-2003). The comparison of means was based on the least significant difference test (Gomez & Gomez, 1984). The correlation between root traits and shoot dry weight, and between root length and root dry weight derived from hydroponics and RLD derived from field conditions were determined using simple correlation.

RESULTS AND DISCUSSION

Root Traits Under Hydroponic Conditions

The eight sugarcane cultivars were significantly different in all root traits, such as root length, root surface area, root volume and root dry weight. KK3, TBy27-1385 and KPS01-12 cultivars represented a group with robust root characters, with higher values for root length, root surface area, root volume and root dry weight. In contrast, CSB06-5-20 and NSUT08-22-3-13 were identified as the small root system cultivars, with low values for all the root traits evaluated (Fig. 3 and Fig. 4).

The main function of roots is to absorb water and inorganic nutrients, thus support the physiological processes (Henry, 2013; Mathieu, Lobet, Tocquin, & Périlleux, 2015), and may indirectly contribute towards accumulation of organic substrates, growth and dry matter accumulation (Robertson, Inman-Bamber, Muchow, & Wood, 1999). A large root system is a key trait for drought resistance in sugarcane (Jangpromma, Thammasirirak, Jaisil, & Songsri, 2012) since under pot conditions, high root dry mass, root length density, root surface area and root volume were directly associated with a drought-resistant genotype (Jangpromma, Thammasirirak, Jaisil, & Songsri, 2012). Additionally, the root traits of sugarcane were shown to vary with different genotypes under pot (Wagih, Ala, & Musa, 2003) and field (Ohashi, de Matos Pires, Ribeiro, & de Oliveira Silva, 2015) conditions.

Fig. 3. A comparison of (a) root dry weight, (b) root length, (c) root volume and (d) root surface area of eight sugarcane cultivars at 3 months after transplanting, under the hydroponic system. Standard error difference of means was shown via vertical bars and mean with the same letters are not different by LSD at p < 0.05.
Despite there was no root study experiment in hydroponic conditions, this current result was supported by rice (Rajkumar & Ibrahim, 2014) and wheat (Ayalew, Ma, & Yan, 2015) as hydroponic can classify the difference of root traits with diverse genotypes. In the current study, KK3 and KPS01-12 had high root length, root dry weight, root surface area and root volume. Consequently, these cultivars were classified as excellent performers with respect to the root system. Root performance of the two cultivars might induce drought resistance, as identified in previous reports by Jangpromma, Thammasirirak, Jaisil, & Songsri (2012) and OCSB (2014).

**Relationship Among Root Traits Under Hydroponic Conditions**

Among the root traits assessed in this study, all paired root traits were positively correlated (Table 1). Root dry weight was the easiest trait for sample collection, and it was related to root volume, root surface area and root length ($r = 0.75^*, 0.87^{**}$ and $0.93^{**}$, respectively). Root length and root surface area revealed a correlation value of $0.96^{**}$, and root surface area was positive correlation with root volume ($r = 0.96^{**}$). Thus, root dry weight might be an appropriate trait for sugarcane root investigation under hydroponic conditions, since it had lower correlation values with root surface area and volume.

In addition, all paired root characteristics in this study were positively correlated. Despite no prior report regarding the relationship among root traits of sugarcane under hydroponic conditions, root characteristics, namely root length, root surface area and root volume, of *Lupinus angustifolius* (Lupin) under hydroponic conditions had correlation values in the ranges of 0.80 - 0.97 (Chen, Dunbabin, Diggle, Siddique, & Rengel, 2011). Similar to the root traits, hydroponics screening helps to classify diverse cane lines based on the differences in above-ground traits. In various lettuce cultivars, this method provided an effective means of controlling the nutritional conditions, to compare the above-ground growth and to classify the shoot fresh weight (Genuncio,}

**Table 1. Correlation among root traits at 3 months after transplanting under the hydroponic system**

<table>
<thead>
<tr>
<th>Root length</th>
<th>Root surface area</th>
<th>Root volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root surface area</td>
<td>0.96 **</td>
<td>Root volume</td>
</tr>
<tr>
<td>Root volume</td>
<td>0.86 **</td>
<td>0.96 **</td>
</tr>
<tr>
<td>Root dry weight</td>
<td>0.75 *</td>
<td>0.87 **</td>
</tr>
</tbody>
</table>

Remarks: *, ** = significant at 5, 1 % level, respectively.

As mentioned above, several previous reports demonstrated that sugarcane genotypes vary in their growth and physiological above-ground characteristics under pot (Jangpromma, Songsri, Thammasirirak, & Jaisil, 2010) and field (Singkham et al., 2016; Smit & Singels, 2006) conditions.

**Above-ground Traits Under Hydroponic Conditions**

Differences in all the above-ground characteristics studied, including stem height, stem diameter and leaf, and stem and shoot dry weight, were observed among the eight sugarcane cultivars. The cultivars which exhibited superior root traits also showed superiority for shoot traits. TBy27-1385 showed high values for plant height, leaf, stem and shoot dry weight and stem diameter, whereas KK3 revealed superior leaf dry weight and large stem size. KPS01-12 showed substantial leaf dry weight and stem diameter, and these parameters contributed to high shoot dry weight. Conversely, the small root system cultivars, such as CSB06-5-20 and NSUT08-22-3-13, had low values for these above-ground traits. CSB06-5-20 had low leaf, stem and shoot dry weight, and NSUT08-22-3-13 performed poorly in stem dry weight and stem height (Fig. 5).

Due to limited references on sugarcane response under hydroponics, the information from other crops to support this result are needed. In the same monocotyledon plants such as rice and wheat, shoot characteristics were varied with different cultivars when grown under hydroponics (Rajkumar & Ibrahim, 2014; Raziuddin et al., 2010). The growth of above-ground plants parts is strongly dependent on the developmental stage of the roots, and the size of both parts might be inter-related, as large shoots are linked to large root systems (Gregory, 2006).

**Fig. 5.** A comparison of (a) stem height, (b) leaf dry weight, (c) stem diameter, (d) shoot dry weight and (e) stem dry weight of eight sugarcane cultivars at 3 months after transplanting, under the hydroponic system. Standard error difference of means was shown via vertical bars and mean with the same letters are not different by LSD at p < 0.05

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In sugarcane, the root system might closely correlate with biomass, one of the main traits related to drought resistance, as reported by Jangpromma, Thammasirirak, Jaisil, & Songsri (2012). Besides, drought could also interrupt cell division and elongation (Machado et al., 2009). Shoot mass and elongation are more highly affected by water-deficit than root (Inman-Bamber, Bonnett, Spillman, Hewitt, & Jackson, 2008; Smit & Singels, 2006). In small pot studies, root traits, such as root dry weight, root volume and root surface area of sugarcane, did not relate to the above-ground part after 10 days of drought (Jangpromma, Thammasirirak, Jaisil, & Songsri, 2012), which conflicts with the current result, possibly due to the limitations of pot space and drought conditions.

**Relationship Between Root and Shoot Traits Under Hydroponic Conditions**

Shoot dry weight, including leaf and stem dry weight of the eight sugarcane cultivars, was positively correlated with root dry weight, root volume and root surface area, but not root length (Fig. 6). Thus, it might indicate that these root traits contributed to the dry weight accumulation of the above-ground parts. Under hydroponic conditions, leaf dry weight was positively related to all root traits, such as root length ($r = 0.76^*$), root surface area ($r = 0.77^*$), root volume ($r = 0.88^{**}$) and root dry weight ($r = 0.89^{**}$). Stem diameter showed a high and significant correlation with root surface area ($r = 0.79^*$) and was highly associated with root volume and root dry weight ($r = 0.84^{**}$ and $0.87^{**}$, respectively). Also, stem and root dry weight were strongly related ($r = 0.78^*$) under hydroponic conditions. Nevertheless, there was no correlation between stem height and any of the root traits, indicating that root were not a key trait which contributes to cane height in this hydroponics experiment.

**Fig. 6.** Relationship between root traits and shoot dry weight of eight sugarcane cultivars at 3 months after transplanting, under the hydroponic system
In addition, the correlation between root and shoot traits had lower than relationship among root traits. Related study has also been reported in rice which are in monocot, the root system development was positively correlated with shoot mass production under water insufficient conditions (Kano, Inukai, Kitano, & Yamauchi, 2011). Root characteristic could encourage growth, yield and productivity of sugarcane under drought conditions, due to its pertinent roles on many physiological processes, like transpiration, water status in leaf and photosynthesis (Khonghintaisong, Songsri, Toomsan, & Jongrungklang, 2018).

**Root Length Density (RLD) Under Field Conditions**

A difference in RLD in the field experiment was observed among the eight sugarcane cultivars. The sugarcane genotypes which are used in this study could be classified into 3 groups, consisting high (ranged 0.15-0.26 cm/cm³), medium (ranged 0.10-0.12 cm/cm³) and low (ranged 0.07-0.08 cm/cm³) RLD value groups. KK3, KPS01-12, CSB06-4-162 and KK07-478 cultivars represented the high value of RLD. In contrast, CSB06-5-20 and NSUT08-22-3-13 were identified as low values in RLD, whereas TBy27-1385 and CSB06-2-15 represented the medium RLD group (Fig. 7). Water deficit at formative phase definitely affected root length of sugarcane under field (Madhav, Bindu, Kumar, & Naik, 2017) and pot (Khonghintaisong, Songsri, Toomsan, & Jongrungklang, 2018) conditions, while the response of root length depended on genetic variations (Khruengpatee, Khonghintaisong, Songsri, & Jongrungklang, 2018). Root was the first structure to expose and signal the moisture lacking in the soil to other cells, tissues and organs, thus understanding the activity of gene in root of sugarcane could develop strategies to improve sugarcane yield (Ferreira et al., 2017).

**Relationship Among the Root Traits in Field and Hydroponics**

The cultivars which exhibited desirable root system performance under hydroponic conditions also showed high values for RLD in field test. KK3 and KPS01-12 had high RLD values under field conditions, whereas TBy27-1385 had a low RLD. The small root system cultivars such as CSB06-5-20 and NSUT08-22-3-13 under hydroponics, had low values of RLD in field test (Fig. 8). Although the methodology for field measurements and that under hydroponics varied, root length from hydroponic experiments was positively correlated to RLD from field conditions ($r = 0.76^*$), and root dry weight in hydroponics was positively correlated to RLD in field condition ($r = 0.72^*$) (Fig. 8).

![Root length density (RLD) of eight sugarcane cultivars at 4 months after transplanting, under field conditions](image)

Fig. 7. Root length density (RLD) of eight sugarcane cultivars at 4 months after transplanting, under field conditions
Again, there was limited reports regarding the relationship between root traits of sugarcane under hydroponic and field conditions. Under hydroponic system, the root characteristic of peanut was positively correlated with that when grown under potted conditions (Girdthai et al., 2010). Correlation between root traits in hydroponics and soil medium were also reported in wheat (Mian, Nafziger, Kolb, & Teyker, 1993) and cowpea (Ogbonnaya et al., 2003). Therefore, hydroponic cultivation is possibly an alternate method for root investigations, as it allows classifying the difference in root and shoot traits.

Fig. 8. Relationship between root length (RL) (a) and root dry weight (b) derived from hydroponics and root length density (RLD) derived from field conditions of eight sugarcane cultivars.

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among sugarcane lines. Moreover, relationships exist between shoot and root traits, and between root length derived from hydroponic and RLD derived from field conditions. This information might be useful for further drought-resistant studies in both breeding and physiological aspects, for identifying reliable related traits that could be used for drought resistance breeding, and also for investigating nutrient use efficiency in diverse sugarcane genotypes.

**CONCLUSION**

Root and shoot traits of sugarcane lines grown under a hydroponic system were significantly different. KK3 was defined as a cultivar having high root dry weight, RLD, root volume and root surface area, and TBy27-1385 performed well regarding the shoot aspects. Moreover, root dry weight, root volume and root surface area had a positive correlation with above-ground dry weight. The trait relationships were also detected between root traits i.e. root length and root dry weight derived from hydroponic and RLD from field conditions. Thus, hydroponic cultivation could be an alternative approach for root and shoot traits investigation in sugarcane.

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