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Responses of rooting and physiological characteristics of sugarcanes grown under mimic drought stress as low water potential at early stage

Wasuthorn Buakom[1], Puntiwa KrachaI[2], Santimaitree Gonkhamdee[1,3] , Patcharin Songsri[1,3] and Nuntawoot Jongrungklang[1,3*]

ABSTRACT: Variation of physiological and morphological response is often caused by different soil environments and conditions. Therefore, better understanding of physiological responses and root attributes of sugarcanes subjected under uniform, controllable condition is encouraged. This study aimed to investigate the responses of rooting and physiological traits of sugarcanes under mimic drought stress as low water potential via PEG induction at early growth stage in hydroponics. Experiment was laid out in split-plot in randomized complete block design (RCBD) with four replications under hydroponic system. The effect of two PEG levels (0.0% and 1.0%) was placed as main plot, whereas sub-plot was the the four sugarcane genotypes. Data was recorded on physiological, morphological, and rooting traits as time series during the periods of transplanting to 3 months after planting (MAP). In general, four sugarcane genotypes grown under control conditions showed higher dry weight, height, leaf area, leaf number, root length, root surface area, root volume, SPAD chlorophyll meter reading (SCMR) and Chlorophyll fluorescence (fv/fm) than those grown under PEG treatment. KK3 cultivar contributed to the root proportion into deeper layer (20-40 cm) when subjected to PEG treatment. Photosynthesis was decreased due to reduced stomatal conductance, as a mechanism to decrease CO_2 exchange rate. The response of photosynthesis, transpiration rate, and leaf area correlate to the performance of sugarcane biomass in response to low water potential via PEG induction under hydroponics. This information provides a basic knowledge for further against drought stress work.
Keyword: root length, photosynthetic rate, biomass, polyethylene glycol, hydroponics

Introduction

The major production areas of sugarcane (*Saccharum officinarum* L.) are rain-fed condition (Siebert and Döll, 2010) that are typically prone to experiencing water deficits. The condition such soil dehydration can significantly reduce sugarcane yield up to 80% (Singh and Rao, 1987), constraining cane production. In Thailand, farmers mostly start growing sugarcane in the late rainy season, meaning that the germinated seedlings may encounter early water deficit (Khonghintaisong et al., 2018). The use of drought-tolerant sugarcane cultivars is commonly applied as strategy to solve this obstacle by drought avoidance and reducing dehydration injury (Medeiros et al., 2013). Therefore, better understanding on drought mechanism including how plant could deal with the water deficit and what the drought-related traits as parameter in indirect selection is necessary to develop drought-tolerant sugarcane genotypes.

The responses of physiological and morphological traits of sugarcane cultivars are recognized as selection criteria for drought-tolerant selection (Smit and Singels, 2006). SCMR, Fv/

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[1] Department of Agronomy, Faculty of Agriculture, Khon Kaen University, Muang, 40002 Khon Kaen, Thailand
[2] Faculty of Science and Technology, Loei Rajabhat University, Loei Province, 42000
[3] Northeast Thailand Cane and Sugar Research Center, Khon Kaen University, Muang, 40002 Khon Kaen, Thailand
*Corresponding author: nuntawootjrk@gmail.com

Fm and photosynthesis are criteria to study on drought (Jangpromma et al., 2010; dos Santos and Silva, 2015; Khonghintaisong et al., 2018). In addition, root attributes play an important role for drought tolerance. For instance, root length, root surface area, and root dry weight are identified as selection criteria for drought tolerance in sugarcane (Jangpromma et al., 2012; Khonghintaisong et al., 2018); however, genotypic variation existed in response to root traits (Khonghintaisong et al., 2018). The physiological traits of sugarcane such as SPAD chlorophyll meter reading (SCMR) and chlorophyll fluorescence also reduced when subjected to water-limit field conditions (Silva et al., 2011; Zhao et al., 2012; Begum et al., 2012; Jaiphong et al., 2016); however, these parameters did not respond well to water deficits in pot trials (Khonghintaisong et al., 2018). The divergent patterns of plant response to drought may be due to different both soil environments and conditions. Therefore, the understanding of physiological responses in relation to below-ground root attributes of sugarcane genotypes subjected under uniform, controllable condition needs to be established.

The root expression is restricted by the soil compaction limiting the response of root plasticity and yield (Correa et al., 2019; Morris et al., 2017). Root studies under soil media are not only difficult but also time consuming, laborious, and costly (Girdthai et al., 2010). Multiple steps including root sampling from soil and root cleaning are required and often result in sample losses and errors (Chapae et al., 2019). Planting using soilless media is a technology that can effectively control various soil-related factors (Wahome et al., 2011; Trejo-Téllez and Gómez-Merino, 2012). This method guarantees users about the uniformity of nutrient application and free plant-disturbing organisms such as weed, pest, and disease. Currently, hydroponics is one of the standard methods that are widely used in both commercial and industrial applications (Shavrukov et al., 2012; Nguyen et al., 2016). In sugarcane, associations among root traits were positive, i.e. root length and root dry weight derived from hydroponics, and root length density (RLD) from field conditions (Chapae et al., 2019). Also, hydroponics could identify genotypic variability regarding different cultivars response on some root attributes either at vegetative stage 3 MAP (Chapae et al., 2019).

Polyethylene glycol (PEG) is commonly used to reduce the osmotic potential under hydroponics; thus, plants are induced to experience dehydration. The use of PEG as drought-inducing agent has been applied for study of drought stress in various crops such as cabbage (Amist and Singh 2016), *Lemna minor* and *Brassica napus* (Osmolovskaya et al., 2018), wheat (Robin et al., 2015), rice (Hannan et al., 2020), and sorghum (O'Donnell et al., 2013). To the best our knowledge, there is no report on the physiological and rooting responses of certain sugarcane cultivars grown under hydroponics with PEG treatment to induce low water potential condition. Therefore, this current study aimed to investigate the responses of rooting and physiological traits of sugarcane genotypes under mimic drought stress as low water potential via PEG induction at early stage in hydroponics. This information will be useful for further against drought stress work by providing a basic knowledge of drought-tolerant mechanisms.

Materials and methods

Plant materials

Four sugarcane genotypes with different root responses to water deficit were used in this study. One commercial sugarcane variety KK3 was identified as drought-tolerant cultivar in Northeast of Thailand and had a good performance on rooting traits (Jangpromma et al., 2012; Khonghintaisong et al., 2018; Chumphu et al., 2019; Khonghintaisong et al., 2020). Genotype UT13 derived from wild-type genotype (Palachai et al., 2019; Khonghintaisong et al., 2020) was assumed as the drought-tolerant cultivar and had a long root length density both in the deep and top-soil (Chumphu et al., 2019). Genotype UT12 had the high root length density in sub-soil layer (Chumphu et al., 2019) and was identify susceptible to drought (Khonghintaisong et al., 2020) and mainly planted in irrigated areas. Genotype KPS01-12 was suitable for sandy soil and had high root length density both in the top and sub-soils (Chumphu et al., 2019), good adaptation, and high cane yield (Palachai et al., 2019; Khonghintaisong et al., 2020).

Experimental design

Four sugarcane genotypes were evaluated under hydroponics system in controlled greenhouse, Agronomy Field Crop Station, Khon Kaen University, Thailand during April to July 2019. The experiment was laid out in split-plot design in RCBD with four replications. The effect of PEG with two PEG concentration levels (0.0% and 1.0%) was assigned as main plot, whereas the effect of sugarcane genotype with four levels of cultivar (KK3, UT13, UT12, and KPS01-12) as sub-plot.

Crop management in hydroponics

Stem of each sugarcane genotypes was cut around 3-4 cm long. Then, these cut stems were planted in each plastic nursery bag 8.5 × 10 cm. Sugarcane seedlings were then transplanted into a hydroponic system at 2 weeks after planting (at least had 3-4 leaves) (Chapae et al., 2019; Chapae et al., 2020). The hydroponic system was filled with water at the potential of hydrogen at 6.2-6.4 with an electrical conductivity (EC) of 0.6-0.8 ds/m. Sugarcane seedlings were planting in pot with plastic grille depth level as 0-20, 20-40 and 40-60 cm under each pot. The Dynamic Root Floating Technique (DRFT) method was applied in the hydroponic for aeration system and have water flow is maelstrom from main tank to each pot, then the solution form each pot will flow back to main tank every 24 h. The nutrient solution for plant fertilizer had two formulas, namely formula A and B. For instance, formula A composed of 50 L water, 5.5 kg $Ca(NO_3)_2$ (Calcium Nitrate), and 80 g Ferric-EDTA. While, formula B composed of 50 L water, 435 g $NH_4H_2PO_4$ (mono-ammonium phosphate), 5 kg KNO_3 (Potassium Nitrate), 2.82 kg $MgSO_4$ (Magnesium Sulphate), 875 g KPO_4^-, 9 g Mn-EDTA (Manganese), 5.5 g Zn-EDTA (Zinc), and 2 g Cu-EDTA (Copper) (Chapae et al., 2019 and Chapae et al., 2020). During the experimental period, the EC was checked and monitored every three days for maintaining the nutrient concentration, the nutrient solution of A and B was 4 ds/m

For control treatment, the solution media was not treated with PEG throughout the period of study, and water level was adjusted to add in the solution as the same content with that of PEG treatment. For PEG treatment, the osmoticum was applied at 35 day after planting (DAP), and the concentration was gradually increased every 7 days interval from 0.25% (at 35 DAP), 0.50% (at 42 DAP), 0.75% (at 49 DAP) and 1.00% (at 56 DAP) of PEG concentration. Recovery period is the duration of plant to regain well-water condition from 63 DAP until reaching 84 DAP.

Data collections

1. *Morphological growth data*

The non-destructive sampling method was applied to record morphological growth traits at above ground such as tiller number per plant, stem height, and leave number of main stem per plant. Meanwhile, the destructive sampling method was performed to measure dry stem weight per plant, leaf dry weight per plant, leave number of main stem per plant and, total leaf area per plant. The tiller number per plant was counted including the first cane stem that has been appeared, and it was observed monthly at one, two, and three MAP. The stem height was measured at the main stem from the water surface until dewlap point, and it was observed since 1 MAP to 3 MAP with seven days interval. The leaf area was determined by LI-3100C area meter at 3 MAP only. Whole plant parts namely leaf, stem, and root were separately heated in the oven at 80 °C around 72 h. Then, these dried samples were measured with analytical balance for dry stem weight per plant and dry leaf weight per plant at 3 MAP.

2. *Physiological traits*

Leaf water potential (LWP) was measured at 41, 48, 55, and 62 DAP when each different PEG levels had been applied, viz in first interval form 0.25% (at 35 to 41 DAP), 0.50% (at 42 to 48 DAP), 0.75% (at 49 to 55 DAP) and 1.00% (at 56 to 62 DAP) of PEG concentration. The value was recorded with pressure chamber/bomb (Model 3005F01, Soil moisture Equipment Corporation, Santa Barbara, California, USA) between 10.00 a.m. to 1.00 p.m. on leaf apex in each pot both under control treatment and PEG treatment.

Chlorophyll fluorescence (Fv/Fm), SPAD chlorophyll meter reading (SCMR) was observed interval of 1 to 3 MAP, Fv/Fm was observed between 10.00 a.m. and 12.00 a.m. at the bottom, middle, and tip of the 2nd fully extended leaf from

the top of main stem with PAM-2000 Heinz Walz GmbH, Germany. SCMR was observed with SPAD-501, Minolta, Tokyo, Japan between 9.00 and 12.00 a.m. on the same sample as Fv/Fm.

Photosynthetic rate, transpiration rate, and stomatal conductance was observed interval of 2 and 3 MAP to H_2O was observed monthly at 2 and 3 MAP with LI-COR (LI-6400 XT Portable Photosynthesis System) between 10.00 and 12.00 a.m. on the same sample as Fv/Fm and SCMR.

3. *Root traits*

The attributes of root including root dry weight, root length, root volume, and root surface area were separately collected at 0-20, 20-40, and 40-60 cm root depth. The root samples were scanned with Epson perfection V800 photo scanner, and scanned samples were analyzed with WinRhizo program (WinRhizo Pro (s) V. 2004a, Regent Instruments, Inc.) to determine root length, root volume, and root surface area. The root samples were further oven-dried at 80 °C for 72 h or constant weight, and root dry weight was measured.

Statistical analysis

The measurement data were subjected to analysis of variance, according to a split-plot in RCBD. The genotype mean was compared with Least Significant Difference (LSD) test at 5% (Gomez and Gomez, 1983). Statistix 10.0 software was computed to facilitate data analysis. The Drought Tolerance Indices (DTI) was calculated for some physiological traits, leaf dry weight (LDW), stem dry weight (SDW) and root dry weight (RDW) at 3 MAP under PEG treatment (stress treatment) to that under control treatments (non-stress treatment), as suggested by (Nautiyal et al. 2002; Songsri et al., 2008), using the relationship as follows:

$$\text{DTI} = \frac{\text{stress treatment}}{\text{non-stress treatment}}$$

Results and Discussion

3.1. Leaf water potential (LWP) of four sugarcane genotypes under hydroponics system.

Leaf water potential of all sugarcane genotypes on two PEG levels were found different (**Figure 1a-d**). The differences of LWP between two PEG treatments increased along with increasing PEG content. While control treatment did not affect the LWP of four genotypes, the increased concentration of PEG from 0.25% to 1.00% significantly reduced the LWP of these genotypes. For instance, decreasing LWP could be noticed on UT13 genotype from -1.0 to -2.0 MPa (Figure 1a), KK3 genotype from -1.0 to -2.3 MPa (**Figure 1b**), Kps01-12 genotype from -1.0 to -2.0 MPa (Figure 1c), and UT12 genotype from -1.0 to -2.0 MPa (**Figure 1d**) along with increased PEG concentration from 0.25% to 1.00%. As mentioned above, LWP of four genotypes was consistently above -0.5 MPa under control treatment, whereas it was stable below -1.0 MPa under PEG. This pattern declared that mimic-drought stress condition for sugarcane genotypes under hydroponics could be established by applying PEG concentration at 0.25% regarding LWP's indicator.

Plant water status is typically described in terms of "water potential," a measure of the free energy status of water relative to pure water at a reference state (Haswell and Verslues, 2015). Leaf water potential (LWP) was used in this study to represent plant water status between control and PEG treatments among sugarcane genotypes under hydroponics. This parameter would be dropped when increased concentration of dissolved solutes or adhesion to hydrophilic surface such as soil particles, whereas it would be inflated when the plant cell experienced increased turgor pressure (Haswell and Verslues, 2015). Previous studies reported the LWP as reliable indicator to demonstrate water status in the plants against drought stress (Yan et al., 2016). In crops , the LWP from 0 to -0.3 MPa was categorized to normal condition, whereas values above -0.4 MPa and between -1.5 to -2.0 MPa were assumed that the plants faced moderate and severe water stress conditions, respectively (Haswell and Verslues, 2015). In another crop, PEG-6000 was used in beach morning glory *Ipomoea pes-caprae* to induce drought stress (Sucre and Suárez, 2011). They noticed that the value of LWP under well-water condition was ranged from -0.3 to -0.8 MPa, but its value significantly to drop below -1.0 MPa (Sucre and Suárez, 2011).

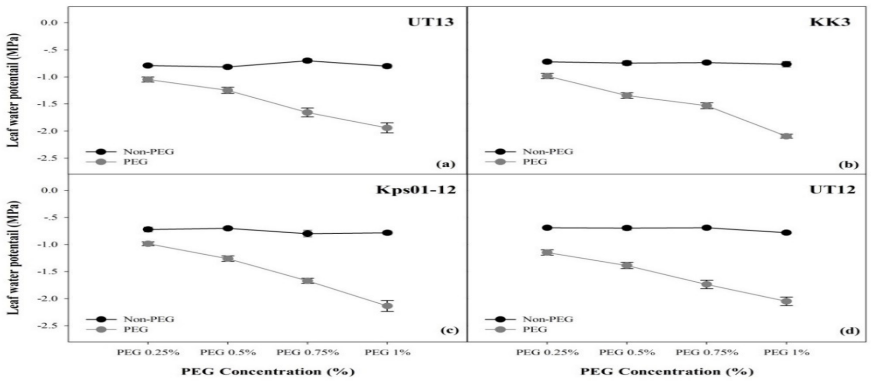

Figure 1 (a-d). Leaf water potential of the two PEG treatments among sugarcane genotypes during PEG applications (at 1-2 months after planting)

3.2. ***Dry weight and growth responses of four sugarcane genotypes under hydroponics system.***

Dry weight characteristics including leaf dry weight (LDW), stem dry weight (SDW), and root dry weight (RDW) of two PEG treatments of all sugarcane genotypes showed different at 3 MAP (**Figure 2**). In general, the highest for dry weight of all cultivars were found in non-PEG treatment, whereas it performed medium dry weight in PEG treatment. Four sugarcane genotypes were divided into 3 groups based on response of dry matter. The 1st group was addressed by UT13 genotype, providing high potential of dry weight in non-PEG treatment but revealing the lowest dry weight in PEG treatment. Consequently, UT13 was defined as high dry weight reduction cultivar indicated by low DTI value of LDW, SDW, RDW and biomass (0.59, 0.58, 0.70 and 0.59 respectively) (**Figure 2**). For the 2nd group, KK3 obtained a good performance of dry weight in both non-PEG and PEG treatments compared with other genotypes. DTI value of LDW, SDW, RDW and biomass of KK3 were 0.74, 0.72, 0.19 and 0.69, respectively, indicating medium reduction cultivars. The 3rd group including Kps01-12 and UT12 cultivars had low dry weight reduction group with medium to high DTI value for these traits ranged from 0.56 to 0.89 (**Figure 2**). This finding revealed the genotypic variability of four sugarcane cultivars on dry weight reduction and DTI in response to drought stress.

The immediate effects of PEG are osmotic stress, using iso-osmotic concentrations of PEG to establish drought stress (Patade et al., 2011). This above finding corroborated previous reports about the effect of PEG reducing dry biomass of both shoot and root in other crops such as in soybean (Hamayun et al., 2010), wheat (Aslam et al., 2018), rice (Larkunthod et al., 2018), and sunflower (Ahmad et al., 2009). In sugarcane, better understanding of plant responses on top and below ground parts will be advantageous for explaining the drought-resistant mechanism (Khonghintaisong et al., 2018). The mechanism of water deficit in sugarcane was that the first reduction occurred in the leaf stalk extension rate then in biomass accumulation and finally in sucrose accumulation (Inman-Bamber et al., 2004). This current study under mimic drought condition by using PEG showed the lowest dry weight of four sugarcane genotypes in comparison to control conditions. This finding reported the similar pattern of plant response to previous investigation in sugarcane under soil media either pot or field condition (Khayatnezhad and Gholamin, 2011; Jangpromma et al., 2012; Khonghintaisong et al., 2018).

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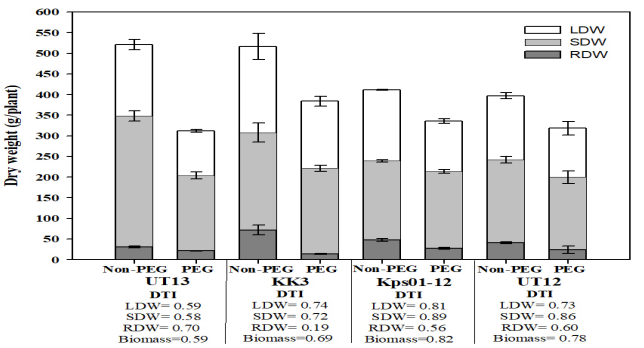

Figure 2 Leaf dry weight (LDW), stem dry weight (SDW) and root dry weight (RDW) of the two PEG treatments among sugarcane genotypes at 3 months after planting (MAP)

In general, tiller number was not different between PEG and non-PEG treatments. Drought stress induced by PEG did not affect the tiller number at either drought period or recovery period for UT13 (**Figure 3a**), KK3 (**Figure 3b**), Kps01-12 (**Figure 3c**), and UT12 (**Figure 3d**). Meanwhile, the stem heights of four sugarcane cultivars namely UT13 (**Figure 3e**), KK3 (**Figure 3f**), Kps01-12 (**Figure 3g**), and UT12 (**Figure 3h**) were significantly suppressed with increased concentration of PEG from 0.25% to 1.00% at 35-62 days after planting and recovery phase (63-84 days after planting).

The above finding about the effect of PEG to tiller number was similar with previous report in sugarcane, and the tillering would be increased sharply after re-watering period (Robertson et al., 1999). The effect of PEG to tiller number has also been reported in other crops with contrasting plant responses. For instance, wheat genotypes responded positively to water deficit by increasing the number of tillers compared to the control treatment (Davidson and Chevalier, 1987). The contrasting results were suspected to be due to the different duration of drought stress given to the plants (Ferreira et al., 2017). In our study, PEG is treated to the plants with seven days interval, and the total drought stress period given was for 28 days; therefore, the not significant effect of PEG to tiller number was caused by short time duration of drought stress and might be due to lower PEG concentration given. The significant suppression of stem height during application of PEG in this study was in contrary with previous reports that PEG did not affect the sugarcane growth (Jangpromma et al., 2012) and stalk height (Khonghintaisong et al., 2018). Previous studies about the effect of PEG showed shoot length reduction in corn hybrids (Khodarahmpour, 2011) and stem height suppression in sunflower (Ahmad et al., 2009).

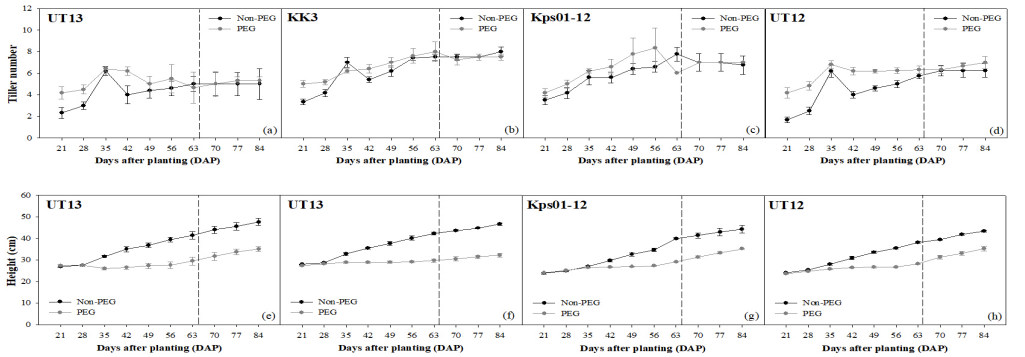

Figure 3 (a-h). Tiller number and stem height at drought period (35-62 DAP) and Recovery period (63-84 DAP) of four sugarcane genotypes

3.3 Root responses of four sugarcane genotypes under hydroponics.

Four sugarcane genotypes grown under control conditions showed higher RL, RSA, and RV than those grown under drought stress at each root depth from 0-20, 20-40, to 40-60 cm. In general, the response of all sugarcane genotype when affecting form PEG under mimic drought condition decrease RL, RSA and RV. The response of root distribution pattern was determined by the change of root percentage in each root depth. Moreover, there tended to occur in parallel among the root traits measured in this experiment.

For UT13 in non-PEG, all root traits including RL, RSA and RV were gradually decreased with more root depth as range 68.48-71.93% (**Figure 4 a-c**) at upper layer (0-20 cm), and 21.44-23.78% (**Figure 4 a-c**) at middle layer (20-40 cm) and 4.29-7.93% (**Figure 4 a-c**) at lower layer (40-60 cm). Whereas in induced drought condition via PEG, rooting traits were shifted to upper layer as 74.04 to 85.64 % (**Figure 4 a-c**), 14.21-25.74% (**Figure 4 a-c**) at middle layer and 0.10-0.20% at lower layer. The root traits of KK3 of control treatment provided at upper layer (86.56-92.81%) (**Figure 4 d-f**) and at middle layer (7.19-13.44%) (**Figure 4 d-f**) but did not found the root at lower layer (**Figure 4 d-f**). Rooting traits in PEG application of KK3 were distributed into upper layer as 86.63 to 87.93% (**Figure 4 d-f**), 11.26-12.21% (**Figure 4 d-f**) at middle layer and 0.12-0.21% at lower layer. For Kps01-12 in non-PEG, all root traits were increasingly decreased with further root depth as range 76.45 - 84.37 % (**Figure 4 g-i**) at upper layer, and 14.92-21.66% (**Figure 4 g-i**) at middle layer and 0.71-1.89% at lower layer. For PEG treatment, rooting traits were contributed to upper layer as 95.45 to 98.99% (**Figure 4 g-j**), 0.66-3.81% (**Figure 4 g-i**) at middle layer and 0.35-0.75% at lower layer. The root traits of UT12 of control treatment provided at upper layer (62.32-76.69%) (**Figure 4 j-l**), at middle layer (17.31-34.99%) (**Figure 4 j-l**) and at lower layer (2.69-6.00%) (**Figure 4 j-l**). Rooting traits in PEG treatment of UT12 were shifted into upper layer as 85.75 to 87.79% (**Figure 4 j-l**), 11.02 -11.36% (**Figure 4 j-l**) at middle layer and 0.92 -3.23% at lower layer. Therefore, in PEG application, KK3 was the one genotype which contributed root proportion into middle and lower layer, but other cultivars had large proportion in upper layer.

The effect of drought on root distribution of sugarcane in field were report by Jangpromma et al., (2012) drought were reduced root length, root surface area, root volume. Support with peanut drought was reduced root traits (Songsri et al., 2009). Similar with our studies, root distribution of sugarcane genotypes in drought conditions were decrease and under mimic drought stress in hydroponic KK3 showed root deeper to lower level. Same with Set-Tow et al., (2020) report the root distribution of KK3 showed both upper and lower soil conditions. Support by de Azevedo et al., (2011) root trait under field conditions was found upper soil layer.

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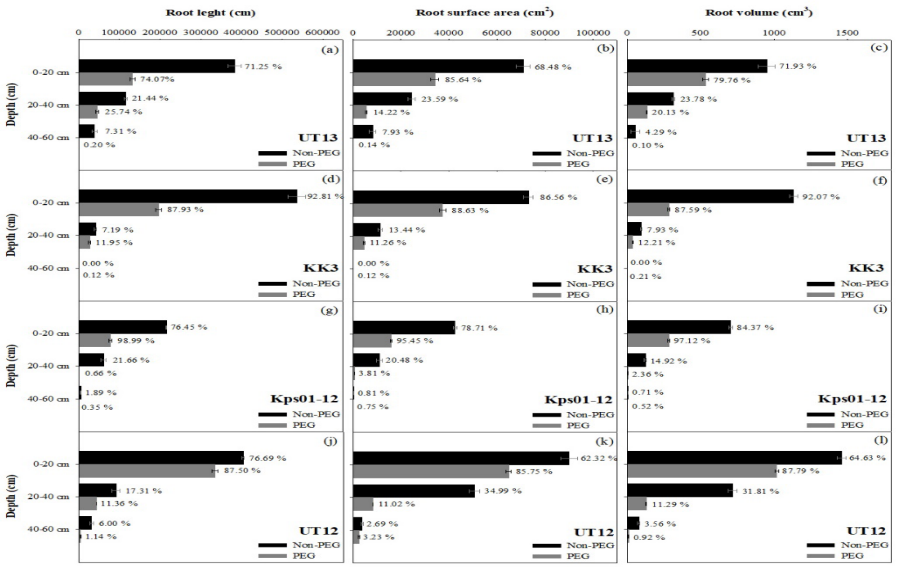

Figure 4 (a-l) The root response root length (RL), root surface area (RSA) and root volume (RV) of four sugarcane genotypes, under non-PEG and PEG treatments at 0-20, 20-40, to 40-60 cm depth at 3 months after planting (MAP)

3.4 Physiological traits and leave responses of four sugarcane genotypes under hydroponics

Leaf number of the sugarcane tested in this investigation were suppressed via PEG. For UT13, PEG reduced the leaf number and continuously affected it in recovery phase (**Figure 5 a**). Leaf number continuously increased under control condition, whereas it was retained after treated PEG for KK3 (**Figure 5 b**), Kps01-12 (**Figure 5 c**) and UT12 (**Figure 5 d**). In addition, four sugarcane genotypes had higher leaf area in control treatment than that in PEG treatment (**Figure 6**). Although these genotypes tested both under control and PEG conditions were ranked in distorted orders, KK3 had the large leaf area when it grown under both conditions for the DTI value 0.45. UT12 had the large leaf area when it grown under control conditions but small area under PEG treated, DTI value was 0.29. UT13 had the medium leaf area under both conditions but small area under PEG treatment, DTI value was 0.34. Kps01-12 had the smallest leaf area under control condition and the slightest leaf area reduction compared to other sugarcane genotypes for the DTI value 0.61 (**Figure 6**). This result indicated that Kps01-12 was the genotype providing low reduction in leaf area, whereas UT13, KK3 and UT12 were experienced high leaf area suppression due to drought stress induced by PEG.

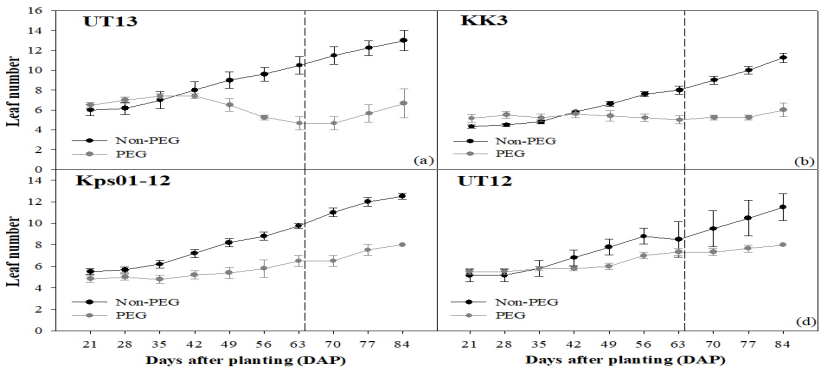

Figure 5 (a-d) Leaf number at drought period (35-62 DAP) and Recovery period (63-84 DAP) of four sugarcane genotypes

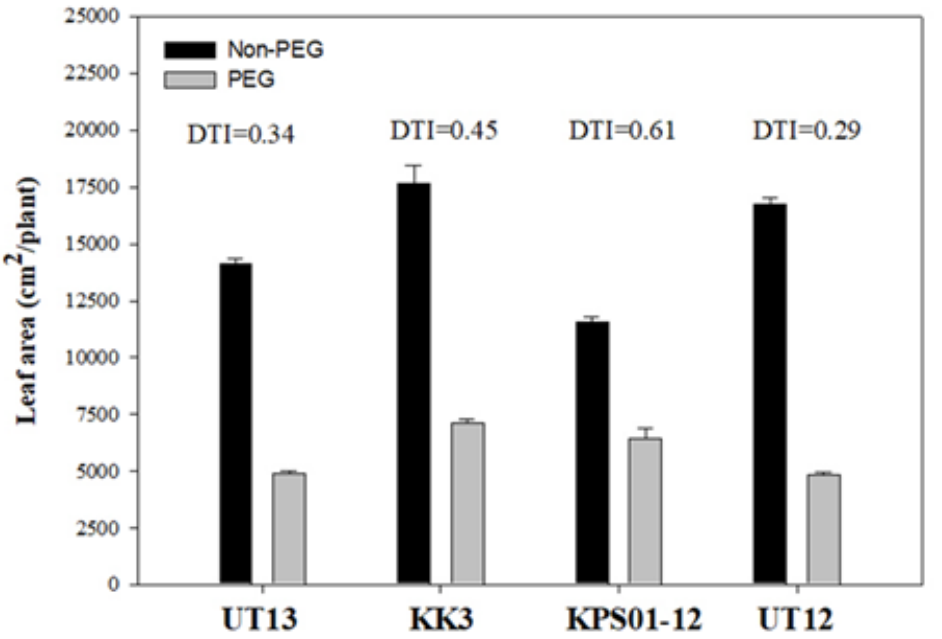

Figure 6 Leaf area at 3 months after planting (MAP) of four sugarcane genotypes

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At 1 MAP chlorophyll fluorescence (fv/fm) of both control and PEG treatments showed similar values of fv/fm, but at 2 and 3 MAP all genotypes under control treatment showed higher fv/fm values than those under PEG treatment (**Figure 7 a-d**). For SCMR, all accessed date (at 1, 2 and 3 MAP) of four genotypes under control treatment were higher SCMR values than PEG treatment (**Figure 7 e-h**). This evidence explained that drought stress induced by PEG under hydroponics clearly decreased these photosynthetic parameters.

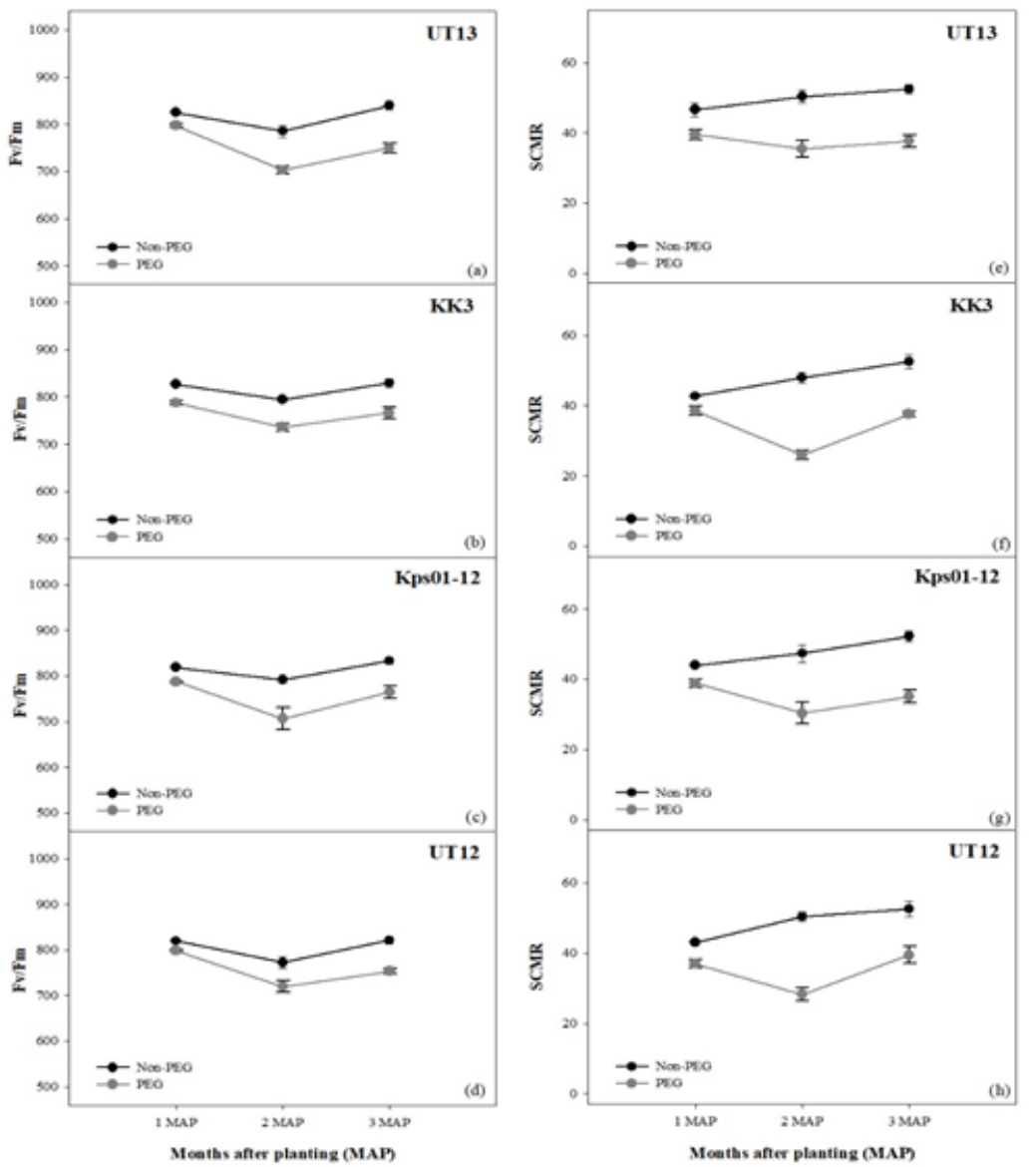

Figure 7 Chlorophyll fluorescence (Fv/Fm) (a-d) and SPAD chlorophyll meter reading (SCMR) (e-h) of four sugarcane genotypes, under non-PEG and PEG treatments

In general, four sugarcane genotypes under control conditions had higher photosynthetic rate than that under PEG treatment. Both photosynthetic rate and DTI value of this trait of four sugarcane genotypes during drought period induced by PEG at 2 MAP differed along with different PEG concentrations given. UT12 showed the lowest reduction of photosynthetic rate with the highest DTI (0.61) (**Figure 8a**). At recovery phase, UT13 was only the genotype that still appreciable reduction of photosynthetic rate, but other genotypes KK3, Kps01-12 and UT12 were able to maintain the photosynthetic rate under PEG treatment compared to non-PEG treatment (**Figure 8b**). This result indicated that KK3, Kps01-12 and UT12 can acclimate photosynthetic rate after recovery, but not for UT13.

Both transpiration rate and stomatal conductance of four sugarcane genotypes during period of PEG application at 2 MAP differed. Four sugarcane genotypes under control treatment had higher transpiration rate and stomatal conductance than those under PEG treatment. UT12 provided low reduction of transpiration rate at 2 MAP, DTI value of UT12, Kps01-12, KK3 and UT13 were 0.52, 0.40, 0.28 and 0.23, respectively (Figure 8c). DTI value of stomatal conductance at 2 MAP for UT12, Kps01-12, KK3 and UT13 were 0.48, 0.30, 0.18 and 0.15, respectively (**Figure 8e**). At recovery, UT13 and Kps01-12 responded to decrease transpiration rate (**Figure 8d**) and stomatal conductance (Figure 8f), but UT12 could maintain these traits under PEG treatment as same with the control treatment. Particularly, KK3 that experienced PEG 1.0% showed higher transpiration and stomatal conductance than control treatment after recovery. Thus, photosynthesis of sugarcane was obviously consistent with the transpirational water loss during osmotic stress.

Physiological parameters are often to use in complement with morphological traits to elucidate sugarcane responses to water deficit (Silva et al., 2011). Water deficit stress alters growth and physiological processes in sugarcane, causing yield loses (Zhao et al., 2010). Drought stress affected to physiological traits of sugarcane in our study. Previous study reported that early season drought reduced the stomatal conductance, chlorophyll fluorescence (Konghintaisong et al., 2018), SCMR (Jangpromma et al., 2010), and photosynthesis rate of sugarcane under pot and field conditions (Zhao et al. 2010). The stomatal conductance became more sensitive under dry soil condition (Smit and Singels, 2006). Another physiological parameter namely transpiration rate also dropped soon during drought period, and it was recovered after receiving re-watering (Medeiros et al., 2013). Inman-Bamber and Smith, 2005 reported that reduced leaf area in sugarcane as indicator to drought adaptation. Also, Endres et al., (2018) informed that the effect of water deficit mostly affected the leaf number of sugarcane during the intense growth phase.

Hydroponics is often used as water media for PEG treatment of various crops. The leave number and chlorophyll content of peanuts were decreasing after raising the concentration of PEG from 5% to 20% (Meher et al., 2018). The increased osmotic stress, induced by PEG, from -0.04 MPa to -1.23 MPa significantly decreased photosynthesis rate, transpiration rate, and stomatal conductance in wheat. Water deficit stress had an impact on the chlorophyll fluorescence parameters of soybean (Zhang et al., 2019). PEG treatments decreased chlorophyll content as compared to control plants (Patade et al., 2011). The physiological response to drought in plant growth was explained by Shao et al., (2008). They explained the consecutive steps during experiencing water deficit stress, as follows: (1) recognition of root signals, (2) loss of turgor and osmotic adjustment, (3) reduced leaf water potential, (4) decreased stomatal conductance to CO_2, (5) reduced internal CO_2 concentration, (6) declined net photosynthesis, and (7) reduced growth rate of plants.

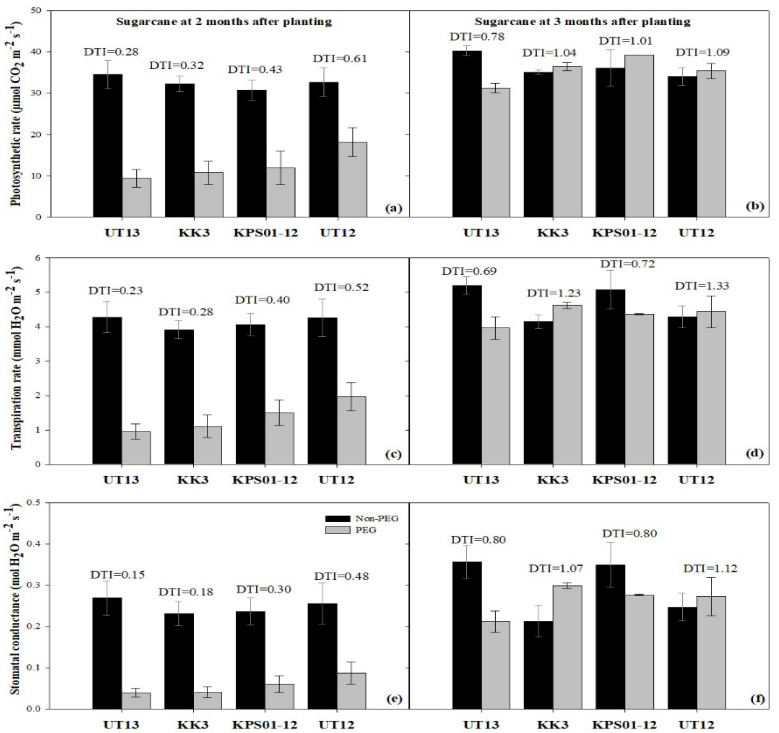

Figure 8 Photosynthetic rate, transpiration rate and stomatal conductance at 2 months after planting (MAP) (a, c, e, respectively) and 3 MAP (b, d, f, respectively) of four sugarcane genotypes, under non-PEG and PEG treatments

3.5 Mechanisms of four sugarcane genotypes under hydroponics at 3 MAP

According to the above results, four sugarcane genotypes were categorized into three groups based on the dry weight reduction i.e. high, moderate, and low reduction. In this study, sugarcane genotypes used alternative mechanisms in response to early drought stress induced via PEG. The responses of physiological parameters such as photosynthetic rate, water loss due to transpiration rate, and leaf area might importantly explain the biomass and related plant growth of sugarcane to such conditions.

The first group was addressed as response to high reduction of biomass. UT13 cultivar, photosynthesis is decreased due to reduced stomatal conductance, as a mechanism to decrease carbon-dioxide exchange rate both stress and recovery periods. Moreover, high reduction of leave number and area under dehydration condition may decrease canopy photosynthesis. Decreasing water loss by reducing transpiration rate and leaf area is a process that can lower biomass under water stress. In this case, the mechanism reduces total dry mass under drought with early growth stage because photosynthesis is greatly affected by drought stress.

The second group, KK3 was defined as medium dry weight reduction when underwent osmotic stress. This could be due to the cultivar provided high reduction in photosynthesis, sto-

matal conductance, and transpiration rate during osmoticum application phase, but it could maintain normal value of photosynthesis during recovery period.

For the last group, Kps01-12 and UT12 responded to low water potential with low biomass reduction. During stress and recovery periods, both two genotypes might use different mechanisms in responses to drought at early growth stage. Kps01-12 showed medium reduction in photosynthesis, stomatal conductance, and transpiration rate during drought duration, and it maintained the value of photosynthesis rate as much as that of control during recovery phase. The response of photosynthesis along with low leaf area reduction of Kps01-12 cultivar may contribute to low reduction of dry mass. Despite UT12 revealed the same physiological response of drought with KK3, it had high DTI of photosynthesis rate during drought stress period. This could lead to high biomass reduction for this genotype.

Drought resistant mechanism consists of two strategies namely maintaining water uptake and reducing water loss. However, it seemed likely that reducing water loss by controlling the transpiration rate was a proper pathway as occurred in UT13, limiting carbon-dioxide influx. This acclimation may be suitable for long-period drought in which the plants are expected to survive in long term period by sacrificing either dry weight or yield. For short-term drought stress, the appropriate mechanism of sugarcane adaptation was to invest more assimilate proportion for supporting root system to above-ground part.

Drought is defined as any restriction of normal functions and development in plants (Ferreira et al., 2017) In order to survive under stress, plants have to follow mechanisms of adaptation namely: (1) escape mechanisms, (2) dehydration avoidance involving mechanisms to retain high water status, and (3) dehydration tolerance referred to as mechanisms allowing plants to tolerate stress (Levitt, 1980). Under severe water stress these mechanisms reduce biomass accumulation through large reduction in transpiration, leaf area and carbon fixation and tolerance traits are directly linked to high stomatal conductance, sustaining the photosynthesis rate (Cominelli et al., 2013). In sugarcane genotype reduced transpirational water loss reduction in stomatal conductance. Consequently, this adaptation mechanism might limit the CO_2 uptake from the air (Konghintaisong et al., 2018)

Conclusion

Artificial drought stress could be established through PEG given under hydroponics, indicated by relative low water potential. All tested sugarcane genotypes grown under non-PEG treatment in hydroponics showed higher dry weight, stem height, leaf area, leaf number, root length, root surface area, root volume, SCMR, and fv/fm than those under PEG treatment. Rooting traits in PEG application of KK3 were distributed into middle layer (20-40 cm) but other cultivars had large proportion in upper layer. Four sugarcane genotypes were categorized into three groups based on the dry weight reduction namely high (UT13 cultivar), moderate (KK3 cultivar), and low reduction (Kps01-12 and UT12 cultivars). Photosynthesis was decreasing due to reduced stomatal conductance, as a mechanism to decline CO_2 exchange rate. The response of photosynthesis, transpiration, and leaf area corroborated the performance of biomass in response to low water potential via PEG induction under hydroponics. This information will be useful for further against drought stress work by providing a basic knowledge of drought-tolerant mechanisms.

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