

Effects of drought and recovery from drought stress on above ground and root growth, and water use efficiency in sugarcane (*Saccharum officinarum* L.)Nisachon Jangpromma¹, Sompong Thammastirak¹, Prasit Jaisil² and Patcharin Songsri^{2,*}¹Protein and Proteomics Research Group, Department of Biochemistry, Faculty of Science, Khon Kaen University, Khon Kaen, 40002, Thailand²Department of Plant Science and Agricultural Resources, Faculty of Agriculture, Khon Kaen University, Khon Kaen, 40002, Thailand

*Corresponding author: patcharinso@kku.ac.th

Abstract

Physiological traits associated with drought tolerance are useful as surrogate traits for drought tolerance. Information on the association of root traits and water use efficiency (WUE) and plant growth in sugarcane is rather limited. The objective of this study was to evaluate genotypic variation for root traits, WUE and growth characters of above ground parts of sugarcane. A 2 × 10 factorial experiment was set up in a randomized complete block design with two replications for three pots per experimental unit in a greenhouse during January to May 2009. Ten sugarcane cultivars were assigned as factor A and two water regimes (well-watered control and water stress at early growth stage) were assigned as factor B. Data were recorded for root length, root surface area, root volume, root dry weight and relative rate of height growth (RHG) at 10 days after dehydration (100 days after planting; DAP) and 10 days after recovery (110 DAP). Data for water use efficiency and root dry weight were recorded at 110 DAP. Stalk diameter was measured at 90 DAP, 100 DAP and 110 DAP. Drought significantly reduced stalk diameter, biomass, root length, root surface area, root volume and root dry weight, but it did not significantly affect root/shoot ratio, WUE, and RHG. Higher phenotypic variations for root traits were observed after recovery and variation of WUE and biomass were also significant. Associations of root traits with biomass and WUE were higher after recovery than before initiation of drought and at drought. The genotypes 03-4-425 and Phill66-07 had higher WUE and large root systems. Root traits are useful as surrogate traits for WUE in sugarcane.

Keywords: Drought tolerance; Genotypic variation; Rehydration; Surrogate trait; WUE.**Abbreviations:** β-Light transmission coefficient measured depending on crop cover; BIO-Above ground biomass; DAP-Days after planting; D-Soil water drainage; DW-Dry weight; E_o-Evaporation from class A pan (mm/day); E_s-Surface evaporation; ET_{crop}-Crop water requirement (mm/day); ET-Evapotranspiration; ET_o-Evapotranspiration of a reference plant under specified conditions calculated by pan evaporation method; FC-Field capacity; FW-Fresh weight; K_c-Crop water requirement coefficient for sugarcane; LSD-Least Significant Difference; M_f-Soil moisture at final harvest; M_i-Starting soil moisture before sowing; PWP-Permanent wilting point; RHG-Relative rate of height growth; R-Surface runoff; t-Days from the last irrigation; WUE-Water Use Efficiency.**Introduction**

Sugarcane (*Saccharum officinarum* L.) is an important crop for sugar production in the tropics, where drought is a recurring problem, and the crop is dependent mostly on rainfall. Drought problems exist in most sugarcane production areas in the world. In Thailand, the largest sugarcane production areas are in the Northeast. These areas usually have lower rainfall than other parts of the country, and sugarcane is highly at risk of drought problems. Drought can reduce sugarcane yield by up to 60% (Robertson et al., 1999). Growing sugarcane cultivars with drought tolerance can alleviate drought problems and reduce yield losses from drought stress. Improvement of drought tolerance in sugarcane is an economical way to improve crop productivity under drought conditions. However, the improvement of drought tolerance in this crop will not be possible if the mechanism underlying the tolerance of the crop is not fully understood. Better understanding of the mechanisms underlying drought tolerance and breeding of crop plants for drought tolerance has been major goals of plant biologists and crop breeders (Xiong et al., 2006). Selection for physiological traits associated with drought tolerance might increase the success of breeding of sugarcane for drought tolerance. The ability of a plant to modify its roots to capture

water for transpiration may be an important mechanism to avoid drought stress (Fukai and Cooper, 1995; Songsri et al., 2009). Plants with high levels of drought avoidance should stabilize yield under drought (Serraj et al., 2004). Root characteristics have been identified as the ability to adapt to drought stress in many plants including peanut (Songsri et al., 2008b), chickpea (Serraj et al., 2004), bread wheat (Gesimba et al., 2004), rice (Wang et al., 2009) and *Arabidopsis thaliana* (Xiong et al., 2006). In sugarcane, Smith et al. (2005) suggested that drought tolerance is higher in cultivars with a tendency to develop deep root systems and found that root system properties may be used as selection criteria for drought tolerance and water use efficiency (WUE). In addition, large root systems can maintain high WUE under drought stress conditions (Songsri et al., 2009). WUE is a ratio of dry matter production to water use (Anyia and Herzog, 2004), and it shows the ability of a crop to convert available water to biomass (Ali et al., 2005). Under drought stress conditions, it would be important to produce a large amount of biomass, which contributes to crop yield, using a low or limited amount of water (Karaba et al., 2007). Therefore, an understanding of the traits associated with WUE such as the ability of roots to increase water uptake and

maintain high photosynthetic capacity should be useful in improving WUE under drought stress conditions. However, information on the relationships between WUE and root traits in sugarcane is rather limited. The objectives of this study were to evaluate genotypic variations of ten sugarcane cultivars and their responses to different water regimes for WUE and root systems, and to determine the inter-relationships among these traits. The information should be useful for a better understanding of the responses of sugarcane cultivars to water stress.

Results and Discussion

Plant and soil water status

Water stress is a common phenomenon and it severely reduces yields of field crops grown under rainfed conditions (Jangpromma et al., 2010a). Therefore, there have been attempts for many decades to identify and understand the traits associated with drought tolerance in sugarcane cultivars. In this study, sugarcane cultivars grown under drought conditions and then allowed to recover from drought were compared with those grown under continuously well-irrigated conditions. Water treatments were clearly different during the stress period (90 to 100 DAP). The stressed plants wilted, whereas the normal plants did not wilt. As wilting occurred in a short period of only 10 days, the stressed plants survived. At 90 days after planting, soil moisture contents were similar for stressed and non-stressed treatments (12.0 and 12.4%, respectively), and the difference between treatments and predetermined value (11.5%) was lower than 1%. The results indicate reasonably good control of the treatments. The details for soil water status determination are reported in a parallel paper (Jangpromma et al., 2010b). After the imposition of drought stress, soil moisture content of the stressed treatment became 3.9%, whereas the predetermined value of permanent wilting point (PWP) was 2.6%. The difference between stressed treatment and PWP was higher than 1% (1.3%), showing that the stressed plants could survive. At 110 DAP when the stressed crop had fully recovered, the soil water status of the stressed treatment was 13%, whereas the soil water status of the non-stressed treatment was 10.5%. The difference of 2.5% might indicate that leaf amount and area of the non-stressed treatment were greater than for stressed plants that had not fully recovered within 10 days. Larger leaves result in higher plant transpiration flux (Verstraeten et al., 2008). Therefore, the non-stressed plants took up a larger amount of soil water than did the stressed plants as the soil of the non-stressed treatment was much dryer than that of the stressed treatment.

Drought and recovery affected root traits, growth parameters and WUE

Although the drought imposition period was very short (10 days), drought significantly reduced root length, root surface area, root volume and root dry weight, but it did not significantly affect the root shoot ratio (Table 1). All root characters could fully recover when the plants were re-watered at 110 days after planting. Drought did not significantly affect water use efficiency (WUE) and relative rate of high growth (RHG), but it did reduce biomass and stalk diameter (Table 2). Biomass and stalk diameter could not fully recover when the plants were supplied with water after withholding. The results indicate that although drought significantly affected root characteristics, these traits recovered rapidly after drought relief. For biomass and stem

diameter, the effects of drought lasted longer and these traits could not fully recover after drought relief. It is expected that if drought lasts longer than 10 days, root traits might not fully recover, and drought could severely affect yield. The results partially explain why drought can reduce yield in sugarcane. The unchanged root/shoot ratio might explain that sugarcane invested in roots under drought conditions to mine more water in deeper soil. Droughts reduced root systems of sugarcane by 50-80%, when the soil water status reduced to a water potential of -0.07 MP (Saliendra and Meinzer, 1992). Most studies have reported drought reduced growth and yield of sugarcane (Inman-Bamber and Smith, 2005; Silva et al., 2008; Smith et al., 2005). The reductions in root traits as affected by drought have been reported in other crops such as in peanut (Songsri et al., 2009), rice (Wang et al., 2009), arabidopsis (Xiong et al., 2006) and sorghum (Tsuji et al., 2005). Drought stress also caused reduction in other drought tolerance traits, for example seed yield (Rahimi et al., 2011), specific leaf area (Jongrunklang et al., 2008; Liu and Stützel, 2004), chlorophyll content (Jangpromma et al., 2010a; Jangpromma et al., 2010b; Silva et al., 2007) and leaf relative water content (Jangpromma et al., 2010a; Silva et al., 2007; Songsri et al., 2008a). However, most reports so far have not explained how plants recover after drought relief. The results of the present study support theoretical expectations and previous results. However, the results provide reasons why drought can affect growth and yield in sugarcane. Significant differences among sugarcane cultivars were observed for root length, root surface area, root volume, root dry weight and root shoot ratio in the stressed period and the recovered period. The cultivar Khon Kaen 3 was the best genotype for these traits at stressed and recovered periods, whereas the cultivar B34-164 performed most poorly for these traits (Table 1). Higher variations in root traits were found at the recovered period. Significant differences among sugarcane cultivars were also observed for WUE and biomass at the recovered period (Table 2). Differences in stalk diameters were not significant at 90, 100 and 110 DAP, whereas differences in relative rate of height growth were found only at 100 DAP. Silva et al. (2008) found high variations among sugarcane genotypes for stalk number, stalk height, stalk diameter, stalk weight and cane yield. The differences in results from different studies could be due to differences in experiment conditions, sugarcane cultivars used in different experiments and timing of sugarcane growth. The results indicate that selection of sugarcane cultivars with good root characters is possible in these sugarcane cultivars, and selection should also be promising for WUE and biomass. The interactions between sugarcane genotypes and water regimes were significant for root length, root surface area, root volume, root dry weight and root/shoot ratio in the recovered period, whereas the interactions for these traits were not significant at the stressed period. The results indicate that sugarcane cultivars show differential responses to re-watering after a stressed period.

Relationships between drought resistance traits and WUE

Under well-watered and drought stressed treatments, the correlation coefficients between root traits and traits related to drought tolerance especially for biomass and WUE are positive but they are still not significant (Table 3). However, most correlation coefficients ($r = 0.65$ to 0.82) become significant in the recovery phase, except for the correlation coefficient ($r = 0.56$) between WUE and root dry weight. It should be noted here that the correlation coefficients between drought related traits (WUE and biomass) and root traits (root

Table 1. Root length (cm), root surface area (cm²), root volume (cm³), root dry weight (g/plant), and root/shoot ratio of 10 sugarcane cultivars at 100 and 110 DAP.

Treatment	Root length (cm)		Root surface area (cm ²)		Root volume (cm ³)		Root dry weight (g/plant)		Root/Shoot
	100 DAP	110 DAP	100 DAP	110 DAP	100 DAP	110 DAP	100 DAP	110 DAP	110 DAP
Soil moisture (A)									
FC	160663 ±78377 A	76794 ±56334 A	16951 ±7603 A	9122 ±6460 A	144.22 ±61.47 A	87.13 ±59.46 A	27.94 ±11.80 A	18.42 ±11.62 A	0.22 ±0.12 A
Stress	116690 ±48893 B	72532 ±29690 A	12104 ±4609 B	8584 ±3134 A	101.22 ±37.39 B	81.61 ±27.24 A	21.42 ±7.46 B	17.69 ±5.56 A	0.24 ±0.07 A
F-test	*	NS	**	NS	**	NS	*	NS	NS
Cultivar (B)									
Uthong 6	145474 ±32302 abc	58438 ±29235 bc	14599 ±2948 abc	6694 ±2826 bc	117.10 ±22.36 bc	61.57 ±21.18 cd	21.03 ±2.77 cde	13.80 ±5.61 cd	0.23 ±0.07 bc
Khon Kaen 80	128893 ±25258 abc	68578 ±31288 bc	13946 ±2064 abc	8599 ±3275 bc	121.36 ±21.23 abc	86.85 ±29.53 bc	24.95 ±3.55 bcd	18.90 ±7.86 bc	0.27 ±0.11 b
K86-161	139598 ±46162 abc	52147 ±23998 bc	15329 ±4085 abc	6706 ±2465 bc	134.91 ±28.44 abc	69.60 ±18.45 bcd	25.03 ±1.85 bcd	15.68 ±4.55 bcd	0.19 ±0.05 bc
Khon Kaen 3	206183 ±92788 a	151747 ±91086 a	21403 ±9611 a	18357 ±9837 a	178.33 ±81.40 a	178.47 ±81.79 a	36.98 ±9.54 a	36.03 ±13.77 a	0.42 ±0.12 a
03-4-425	183443 ±25970 abc	89776 ±20518 b	18304 ±1453 ab	10542 ±2181 b	147.83 ±12.13 ab	99.03 ±17.77 b	33.80 ±5.30 ab	22.55 ±3.07 b	0.23 ±0.03 bc
KU60-1	124107 ±22998 bcd	67991 ±14650 bc	14898 ±3683 abc	8078 ±1800 bc	143.15 ±44.78 ab	76.68 ±18.54 bcd	24.93 ±3.71 bcd	16.48 ±3.33 bcd	0.24 ±0.05 bc
Phill66-07	119199 ±53203 bcd	83196 ±46188 bc	12353 ±5076 bcd	9772 ±4719 b	102.67 ±38.32 bcd	91.69 ±37.79 bc	22.55 ±6.77 cd	19.80 ±7.32 bc	0.22 ±0.10 bc
B34-164	48004 ±14567 d	41206 ±15008 c	5595 ±1755 d	4906 ±1678 c	52.39 ±14.98 d	46.55 ±14.98 d	10.98 ±3.05 e	9.43 ±2.93 d	0.15 ±0.04 c
Uthong 2	87975 ±46008 cd	59282 ±13702 bc	9287 ±4432 cd	6720 ±1678 bc	78.53 ±35.00 cd	61.48 ±7.75 cd	15.25 ±6.18 de	12.60 ±1.89 cd	0.17 ±0.03 c
LF82-2122	203888 ±109174 a	74266 ±24204 bc	19565 ±11229 ab	8156 ±2394 bc	150.94 ±93.98 ab	71.78 ±20.41 bcd	31.30 ±19.03 abc	15.30 ±4.05 bcd	0.19 ±0.04 bc
F-test	*	**	*	**	*	**	**	**	**
A × B	NS	*	NS	**	NS	**	NS	*	NS
CV (%)	38.33	39.93	35.15	33.77	33.67	27.89	29.48	29.75	27.69

NS, *, ** non-significant and significant at 0.05 and 0.01 probability levels, respectively. Means in the same column with the same letters (capital letters for factor A and small letters for factor B) are not different by 95% LSD at $p \leq 0.05$. Each value represents the mean ± SD.

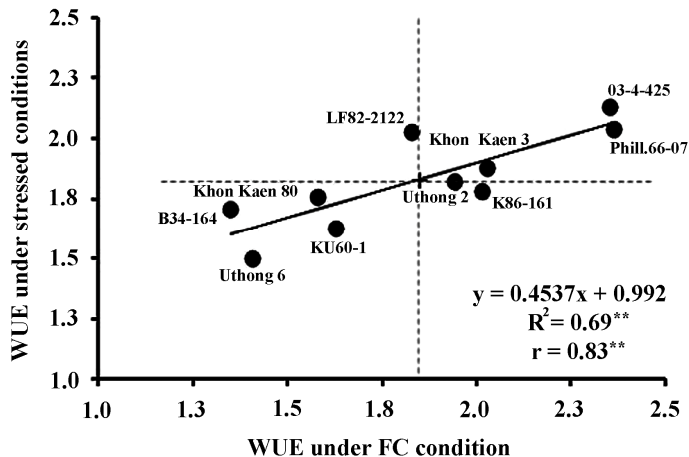


Fig 1. Relationship between water use efficiency (WUE) (g/kg) of 10 sugarcane cultivars under well-watered (FC) and stressed conditions during January to May 2009. ; ** significant at 0.01 probability levels.

length, root surface area, root volume and root dry weight) were much stronger in the drought recovery phase. The results might indicate the importance of roots for growth recovery after drought relief as they can promote flush growth. Traits associated with WUE such as root systems can enhance the yield under water stress (Songsri et al., 2009). Seghatoleslami et al. (2008) reported that proso millet (*Panicum miliaceum* L.) genotype with the greatest seed yield had the highest WUE. Therefore, drought tolerance might be achieved by selection for large root systems that contribute to high yield and WUE under water stress conditions.

Variability of sugarcane cultivars in WUE

Water use efficiency under well-watered conditions ranged from 1.3 to 2.3, whereas WUE under drought stressed conditions ranged from 1.5 to 2.25 (Fig. 1). Therefore, the range of variation in WUE during stress was somewhat lower than that under well-watered conditions. The effect of WUE depends on plant species and severity (Seghatoleslami et al., 2008). Drought may reduce variability in WUE in sugarcane. The correlation coefficients between well-watered conditions and drought conditions were high and significant ($r = 0.83$). The results indicate that the cultivars which showed high WUE under well-watered conditions also showed high WUE under stressed conditions and vice versa. In this case, evaluation under drought stressed conditions may be not necessary. Sugarcane genotypes 03-4-425 and Phill66-07 had high WUE, whereas Uthong 6, KU60-1, B34-164 and Khon Kaen 80 had low WUE, under both drought and well-watered conditions. Higher WUE in 03-4-425 and Phill66-07 was due to higher root systems to capture soil water for transpiration as indicated by high and significant correlation of the traits especially after re-watering (Table 3). In general, crops that have greater WUE are associated with greater yield (Jongrunklang et al., 2008; Karaba et al., 2007; Seghatoleslami et al., 2008; Songsri et al., 2009). Consequently, crops that can maintain high WUE under drought or well-irrigated conditions are considered to be drought tolerant in terms of total dry matter production (Jongrunklang et al., 2008). As water use efficiency was

well-associated with root traits, these traits may be useful for use as selection criterion for WUE.

Materials and methods

Plant culture and stress treatment

The details of the experiment have been described previously (Jangpromma et al., 2010b). Briefly, the stalk at maturity stage (8 months) of ten sugarcane breeding lines and cultivars (Uthong 6, Khon Kaen 80, K86-161, Khon Kaen 3, 03-4-425, KU60-1, Phill66-07, B34-164, Uthong 2 and LF82-2122) provided by the Khon Kaen Field Crops Research Center, Khon Kaen, Thailand were tested for their responses to drought during 90-100 DAP and recovery from drought stress during 101-110 DAP (January to May 2009) at Khon Kaen University. The pot experiment was undertaken under open-sided greenhouse conditions, so that the air temperature was higher than outside by around 2-3°C. Plastic containers 27.5 cm in diameter and 35.0 cm in height were filled with 22 kg of dry soil to create uniform bulk density of 1.5 g/cm³ from the bottoms of the containers to 10 cm below the top of the pots. The soil particles consisted of sand (73.03%), silt (22.67%), clay (4.30%) and organic matter (0.25%), and the soil type was identified as sandy loam. The soil chemical properties comprised pH 5.0, total nitrogen 0.040%, available phosphorus of 72 ppm and extractable potassium at 67 ppm. The soil moisture contents were 11.5% at field capacity (FC) and 2.67% at permanent wilting point (PWP). Sugarcane seed stalks of 10 cultivars were cut into short pieces each of which had one active bud, and they were pre-germinated in germinating trays containing moistened absorbent paper. The uniformly-germinated seed canes were then planted in the plastic containers. There was one plant in each pot for 110 days during January to May 2009. A factorial experiment in a randomized complete block design with two replications was carried out in a greenhouse during January to May 2009 at the Field Crops Research Station of Khon Kaen University located in Khon Kaen province. Two water regimes (field capacity and drought stress followed by recovery) were assigned as factor A and 10 sugarcane cultivars were assigned as factor B. Water was supplied daily to the experiment at field capacity level from planting to 90 days after planting (DAP), and the amount of water was calculated as described previously (Jangpromma et al., 2010b; Songsri et al., 2009). After 90 DAP, water level at field capacity was maintained throughout the experiment for well-watered control. For drought treatment, water was withheld at 90 to 100 DAP. Re-watering was applied after 100 DAP and the stressed treatments were maintained at field capacity. Calculation of total crop water use for each water treatment was calculated as the sum of transpiration and soil evaporation. Crop water requirement was calculated using the equation as described previously (Jangpromma et al., 2010b; Songsri et al., 2009);

$$ET_{\text{crop}} = ET_0 \times K_c$$

where, ET_{crop} is the crop water requirement (mm/day), ET_0 is the evapotranspiration of a reference plant under specified conditions calculated by the pan evaporation method, K_c is the crop water requirement coefficient for sugarcane, which varies with genotype and growth stage. Surface evaporation (E_s) was calculated as described previously (Jangpromma et al., 2010b; Songsri et al., 2009);

$$E_s = \left[\beta \times \left(\frac{E_0}{t} \right) \right]$$

Table 2. Water use efficiency (WUE) (g/kg), biomass (g/plant), stalk diameter (mm.), relative rate of height growth (RHG) (%) of 10 sugarcane cultivars at 90, 100 and 110 DAP.

Treatment	WUE (g/kg)	Biomass (g/plant)	Stalk diameter (mm.)			Relative rate of height growth (%)	
	110 DAP	110 DAP	90 DAP	100 DAP	110 DAP	100 DAP	110 DAP
Soil moisture (A)							
FC	1.85 ±0.37 A	81.59 ±19.80 A	22.53 ±3.88 A	22.98 ±3.49 A	23.93 ±3.31 A	2.82 ±2.88 A	3.60 ±3.33 A
Stress	1.83 ±0.22 A	73.15 ±9.70 B	22.15 ±2.85 A	21.23 ±2.41 B	22.09 ±1.77 B	2.21 ±2.39 A	2.28 ±2.26 A
F-test	NS	**	NS	*	*	NS	NS
Cultivar (B)							
Uthong 6	1.45 ±0.24 e	59.70 ±10.78 d	21.95 ±2.52 ab	22.54 ±2.62 ab	25.11 ±3.99 a	2.70 ±1.24 bcd	3.89 ±3.24 ab
Khon Kaen 80	1.67 ±0.16 de	68.65 ±4.99 cd	22.87 ±3.03 ab	21.69 ±2.53 bc	23.64 ±2.02 a	0.05 ±0.00 e	1.16 ±1.42 b
K86-161	1.90 ±0.22 cd	80.83 ±15.86 bc	24.00 ±1.82 a	23.02 ±1.27 ab	24.63 ±2.88 a	0.79 ±0.95 de	4.26 ±3.41 ab
Khon Kaen 3	1.95 ±0.16 bc	85.20 ±12.40 ab	23.35 ±4.28 ab	25.75 ±4.64 a	24.13 ±3.32 a	6.41 ±3.73 a	4.26 ±3.41 b
03-4-425	2.24 ±0.15 a	98.00 ±12.99 a	21.30 ±1.75 ab	21.98 ±2.62 bc	21.76 ±1.43 ab	3.31 ±2.67 bc	4.39 ±2.45 ab
KU60-1	1.63 ±0.04 e	68.50 ±3.24 cd	21.68 ±1.14 ab	21.55 ±2.84 bc	22.78 ±3.00 ab	0.78 ±0.93 de	1.67 ±0.69 b
Phill66-07	2.20 ±0.22 ab	95.65 ±16.20 a	21.26 ±1.47 ab	22.31 ±2.86 bc	22.49 ±2.18 ab	1.59 ±1.97 cde	3.21 ±4.77 ab
B34-164	1.53 ±0.23 e	60.83 ±8.12 d	19.00 ±2.62 b	18.97 ±2.83 c	19.63 ±1.65 b	4.24 ±3.51 ab	1.21 ±0.47 b
Uthong 2	1.88 ±0.21 cd	76.45 ±11.72 bc	24.40 ±6.94 a	22.17 ±3.94 bc	22.83 ±2.78 ab	1.62 ±0.67 cde	5.38 ±4.36 a
LF82-2122	1.93 ±0.14 c	79.90 ±4.06 bc	23.60 ±4.08 a	21.11 ±2.65 bc	23.11 ±2.32 a	3.68 ±1.95 bc	2.66 ±2.17 ab
F-test	**	**	NS	NS	NS	**	NS
A×B	NS	NS	NS	NS	NS	*	NS
CV (%)	9.14	11.26	13.63	10.43	9.89	66.47	81.03

NS, *, ** non-significant and significant at 0.05 and 0.01 probability levels, respectively. Means in the same column with the same letters (capital letters for factor A and small letters for factor B) are not different by 95% LSD at $p \leq 0.05$. Each value represents the mean \pm SD.

Table 3. Correlation between water use efficiency (WUE), biomass, stalk diameter, relative rate of height growth (RHG), and root traits of 10 sugarcane cultivars under well watered, stress and recovery conditions.

	Root length	Root surface area	Root volume	Root dry weight	Root/ Shoot ratio
<i>FC (110d)</i>					
WUE	0.26	0.27	0.28	0.39	0.22
Biomass	0.34	0.35	0.36	0.47	0.28
Stalk diameter	0.40	0.39	0.39	0.37	0.49
Relative rate of height growth	-0.08	-0.05	-0.02	-0.00	-0.02
<i>Stress (100d)</i>					
WUE	0.38	0.33	0.26	0.35	-
Biomass	0.42	0.37	0.31	0.37	-
Stalk diameter	0.12	0.16	0.17	0.43	-
Relative rate of height growth	-0.31	-0.37	-0.46	-0.02	-0.02
<i>Recovery (110d)</i>					
WUE	0.73*	0.73*	0.67*	0.56	-0.00
Biomass	0.82**	0.81**	0.75*	0.65*	0.17
Stalk diameter	-0.15	-0.15	-0.14	-0.12	-0.34
Relative rate of height growth	0.40	-0.01	-0.13	-0.08	0.24

*, ** significant at 0.05 and 0.01 probability levels, respectively.

where, E_s is the soil evaporation (mm), β is the light transmission coefficient measured depending on crop cover, E_o is the evaporation from class A pan (mm/day), t is the days from the last irrigation. Soil moisture contents were measured by the gravimetric method at 90 DAP, 100 DAP and 110 DAP.

Stalk diameter

The centre point of the stalk was measured for diameter before the imposition of drought (90 DAP), after drought (100 DAP) and at drought recovery (110 DAP) using vernier calipers.

Relative rate of height growth

The relative rate of height growth (RHG) was measured before the imposition of drought (90 DAP), after drought (100 DAP) and drought recovery (110 DAP) according to the method described by Wang et al. (2006);

$$\text{RHG (\%)} = \left[\frac{(\text{final value} - \text{basic value})}{\text{basic value}} \right] \times 100$$

For RHG calculation at drought stress, basic and final values are plant height at 90 DAP and 100 DAP, respectively. For RHG calculation at drought recovery, basic and final values are plant height at 100 DAP and 110 DAP, respectively.

Biomass

After drought recovery for 10 days (110 DAP), the plants were cut at the soil surface and shoot fresh weight (FW) was determined immediately. The plant samples were then oven-dried at 80°C for 48 h and plant dry weight (DW) was determined. Biomass was defined as total above ground plant dry weight.

Water use efficiency

Evapotranspiration (ET) under varying watering regimes was calculated using the soil water balance equation for the growing season as follows:

$$\text{ET} = \text{I} + (\text{M}_i - \text{M}_f) - \text{D} - \text{R}$$

where I = the irrigation applications, M_i = starting soil moisture before sowing, M_f = soil moisture at final harvest (soil moisture was measured by the gravimetric method), D = soil water drainage, and R = surface runoff. Under the pot experiment, soil water drainage and runoff were ignored. Water use efficiency (WUE) for each treatment was calculated as above ground biomass (BIO) divided by seasonal evapotranspiration (ET);

$$\text{WUE} = \frac{\text{BIO(g)}}{\text{ET (kg)}}$$

Root characteristics

After the plants were cut at above ground level, roots were sampled and washed. Ten percent of total roots was used for determination of root length, root surface area and root volume at 10 days after dehydration (100 DAP) and 10 days after recovery (110 DAP). Root samples and shoot samples

were oven-dried at 80°C for 48 h, and dry weights of roots and shoots were recorded. Percentage of root length, root surface area and root volume were calculated using WINRHIZO Pro 2004a software (REGENT Instruments Inc, Quebec, Canada).

Statistical analysis

Analysis of variance was performed for RHG, stalk diameter, biomass, WUE, root surface area, root volume, root dry weight and root/shoot ratio according to a factorial design. Where main effects were significant, Least significance difference (LSD) was used to compare means (Hoshmand, 2006). Correlation coefficients among RHG, stalk diameter, biomass, root surface area, root volume, root dry weight, root/shoot ratio, and WUE were calculated to understand the relationship between drought resistance traits and WUE.

Conclusion

The effects of drought and recovery on 10 sugarcane cultivars were rather similar. Drought caused the reduction of drought tolerance traits and root systems. The sugarcane cultivars 03-4-425 and Phill66-07 were the best genotypes for WUE and large root systems. These might be identified as genotypes having drought avoidance mechanisms. Therefore the root system is the important characteristic associated with WUE. Results of this paper indicate that the response of sugarcane under recovery conditions is best for high WUE. This might be suitable for use as a screening criterion for drought tolerance cultivars. Nevertheless, drought tolerance is a complex trait, the expression of which depends on action and interaction of different morphological, physiological and biochemical characteristics. Hence, in order to understand more about drought tolerance mechanisms in terms of biochemistry, the proteins or genes that are associated with drought should be studied.

Acknowledgments

This research was supported by a grant from the program Strategic Scholarships for Frontier Research Network for the Ph.D. Program Thai Doctoral degree from the Office of the Higher Education Commission, Thailand, the Innovation Potential Research Proposal for High Social Impact Scholarship and the Department of Biochemistry, Faculty of Science, Khon Kaen University. Assistance was also received from the Plant Breeding Research Center for Sustainable Agriculture and Khon Kaen University Research Fund. We are most grateful to Mr. Werapon Ponragdee, Khon Kaen Field Crop Research Center for providing sugarcane cultivars. We thank Mr. Ian Thomas for editing our draft paper.

References

- Ali S, Munir A, Hayat R, Ijaz SS (2005) Enhancing water use efficiency, nitrogen fixation capacity of mash bean and soil profile nitrate content with phosphorous and potassium application. *J Agron* 4: 340-344.
- Anyia AO, Herzog H (2004) Water-use efficiency, leaf area and leaf gas exchange of cowpeas under mid-season drought. *Eur J Agron* 20: 327-339.
- Fukai S, Cooper M (1995) Development of drought-resistant cultivars using physiomorphological traits in rice. *Field Crop Res* 40: 67-86.

- Gesimba RM, Njoka E, Kinyua M (2004) Root characteristics of drought tolerant bread wheat (*Triticum aestivum*) genotypes at seedling stage. *Asian J Plant Sci* 3: 512-515.
- Hoshmand AR (2006) *Design of Experiments for Agriculture and the Natural Sciences*. 2nd Edn., Chapman and Hall, New York.
- Inman-Bamber NG, Smith DM (2005) Water relations in sugarcane and response to water deficits. *Field Crop Res* 92: 185-202.
- Jangpromma N, Kitthaisong S, Lomthaisong K, Daduang S, Jaisil P, Thammasirirak S (2010a) A Proteomics Analysis of Drought Stress-Responsive Proteins as Biomarker for Drought-Tolerant Sugarcane Cultivars. *Am J Biochem Biotechnol* 6: 89-102.
- Jangpromma N, Songsri P, Thammasirirak S, Jaisil P (2010b) Rapid Assessment of Chlorophyll Content in Sugarcane Using a SPAD Chlorophyll Meter across Different Water Stress Conditions. *Asian J Plant Sci* 9: 368-374.
- Jongrunklang N, Toomsan B, Vorasoot N, Jogloy S, Kesmla T, Patanothai A (2008) Identification of peanut genotypes with water use efficiency under drought stress conditions from peanut germplasm of diverse origins. *Asian J Plant Sci* 7: 628-638.
- Karaba A, Dixit S, Greco R, Aharoni A, Trijatmiko KR, Marsch-Martinez N, Krishnan A, Nataraja KN, Udayakumar M, Pereira A (2007) Improvement of water use efficiency in rice by expression of *HARDY*, an *Arabidopsis* drought and salt tolerance gene. *Proc. Nat Acad Sci USA* 104: 15270-15275.
- Liu F, Stützel H (2004) Biomass partitioning, specific leaf area, and water use efficiency of vegetable amaranth (*Amaranthus* spp.) in response to drought stress. *Sci Hort* 102: 15-27.
- Rahimi A, Jahansoz MR, Madah Hoseini S, Sajjadinia AR, Roosta HR, Fateh E (2011) Water use and water-use efficiency of Isabgol (*Plantago ovata*) and French psyllium (*Plantago psyllium*) in different irrigation regimes. *Aust J Crop Sci* 5: 71-77.
- Robertson MJ, Inman-Bamber NG, Muchow RC, Wood AW (1999) Physiology and productivity of sugarcane with early and mid-season water deficit. *Field Crop Res* 64: 211-227.
- Saliendra NZ, Meinzer FC (1992) Genotypic, developmental and drought-induced differences in root hydraulic conductance of contrasting sugarcane cultivars. *J Exp Bot* 43: 1209-1217.
- Seghatoleslami MJ, Kafi M, Majidi E (2008) Effect of drought stress at different growth stages on yield and water use efficiency of five proso millet (*Panicum miliaceum* L.) genotypes. *Pak J Bot* 40: 1427-1432.
- Serraj R, Krishnamurthy L, Kashiwagi J, Kumar J, Chandra S, Crouch JH (2004) Variation in root traits of chickpea (*Cicer arietinum* L.) grown under terminal drought. *F Field Crop Res* 88: 115-127.
- Silva MA, Jifon JL, Silva JAG, Sharma V (2007) Use of physiological parameters as fast tools to screen for drought tolerance in sugarcane. *Braz J Plant Physiol* 19: 193-201.
- Silva MA, Silva JAG, Enciso J, Sharma V, Jifon J (2008) Yield components as indicators of drought tolerance of sugarcane. *Sci Agric* 65: 620-627.
- Smith DM, Inman-Bamber NG, Thorburn PJ (2005) Growth and function of the sugarcane root system. *Field Crop Res* 92: 169-183.
- Songsri P, Jogloy S, Holbrook CC, Kesmla T, Vorasoot N, Akkasaeng C, Patanothai A (2009) Association of root, specific leaf area and SPAD chlorophyll meter reading to water use efficiency of peanut under different available soil water. *Agric Water Manage* 96: 790-798.
- Songsri P, Jogloy S, Kesmla T, Vorasoot N, Akkasaeng C, Patanothai A, Holbrook CC (2008a) Response of reproductive characters of drought resistant peanut genotypes to drought. *Asian J Plant Sci* 7: 427-439.
- Songsri P, Jogloy S, Vorasoot N, Akkasaeng C, Patanothai A, Holbrook C (2008b) Root Distribution of Drought-Resistant Peanut Genotypes in Response to Drought. *J Agron Crop Sci* 194: 92-103.
- Tsuji W, Inanaga S, Arski H, Morita S, An P, Sonobe K (2005) Development and distribution of root system in two grain sorghum cultivars originated from sudan under stress. *Plant Prod Sci* 8: 553-562.
- Verstraeten WW, Veroustraete F, Feyen J (2008) Assessment of Evapotranspiration and Soil Moisture Content Across Different Scales of Observation. *Sensors* 8: 70-117.
- Wang H, Siopongco J, Wade LJ, Yamauchi A (2009) Fractal analysis on root systems of rice plants in response to drought stress. *Environ Exper Bot* 65: 338-344.
- Wang Y, Jiang J, Zhao X, Liu G, Yang C, Zhan L (2006) A novel *LEA* gene from *Tamarix androssowii* confers drought tolerance in transgenic tobacco. *Plant Sci* 171: 655-662.
- Xiong L, Wang RG, Mao G, Koczan JM (2006) Identification of drought tolerance determinants by genetic analysis of root response to drought stress and abscisic acid. *Plant Physiol* 142: 1065-1074.