RESEARCH ARTICLE

GENOTYPIC VARIATION IN ROOT DISTRIBUTION AND PHYSIOLOGICAL RESPONSES OF SUGARCANE INDUCED BY DROUGHT STRESS

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SUMMARY

Drought is an important factor that reduces the yield and quality of sugarcane. Root growth and physiological traits are important for maximizing water uptake in order to improve drought resistance. This study compared the root, shoot, and physiological traits under drought stress (DS) as well as well-watered (WW) conditions of various sugarcane varieties grown in rhizoboxes in a greenhouse. Data were recorded for the following traits: relative water content, stomatal conductance, SPAD chlorophyll meter reading, and chlorophyll fluorescence at 90 days after transplanting. Root samples were recovered from 11 soil layers at 10cm intervals from the top to the bottom of the rhizobox, for root length and root dry weight measurements. Drought was imposed on sugarcane at the early growth stages, which altered the root distribution patterns, creating differences evident among the sugarcane genotypes. The sugarcane genotypes adapted to water stress by increasing root length into deeper soil layers. Drought led to increased total root length in KK3, MPT06-166, K88-92, CP38-22, Kps01-12, and KpK98-40. Root lengths and stomatal conductance were positively correlated under both WW and DS conditions. Root distribution in the lower soil layers and the percentage of root distribution were higher than those under well-watered conditions. The knowledge gained from this study will aid parental selection in sugarcane breeding programs for drought resistance, as the findings strongly suggest that the physiological modification in the root system may be utilized as a useful drought-resistant mechanism.

Key words: Breeding, drought resistance, drought avoidance, root length, rhizobox

Key findings: The adaptation of sugarcane subjected to DS conditions increased root length. This study will aid parental selection in sugarcane breeding programs for drought resistance, as the findings strongly suggest that the physiological

modification in the root system may be utilized as a useful drought-resistant mechanism.

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INTRODUCTION

Sugarcane is a globally important crop since it provides nearly 80% of the sugar consumed worldwide and is cultivated in roughly 130 tropical countries and territories located in the tropics and subtropics all over the world (Sentíes-Herrera *et al*., 2017). Sugarcane is used primarily for sugar production and as an efficient crop for the production of other products, such as electricity, bioethanol, and fertilizer (Unica, 2008). Global sugar production amounted to roughly 178.93 million metric tons (Shahbandeh, 2019). Despite increasing consumer demand for sugar, the cane yield and sugar yield in production systems remain low due to diseases, insect infestations and drought. Most of the sugarcane production areas in Thailand are located in rainfed conditions (Laclau and Laclau, 2009), and drought usually appears during the growing season, especially early season drought (Khonghintaisong *et al*., 2018). Early season drought and mid-season drought can reduce plant growth, resulting in plant stunting and restriction of tillering, leading to vacant and low millable stalk, and losses in both cane yield and sugar yield (Dinh *et al*., 2017). Drought stress can cause yield losses of up to 60% (Robertson *et al*., 1999; Gentile *et al*., 2015). A drought-resistant sugarcane cultivar could maintain yield under both rainfed and drought stress conditions. However, an understanding of drought-resistant mechanisms is a major challenge in sugarcane breeding programs, as drought resistance is inherited genetically, and is associated with physiological characteristics.

Common physiological traits; including leaf area, stomatal conductance, chlorophyll content, relative water content (RWC), photosystem II (PSII), photosynthesis efficiency, and photosynthetic rate have been used to improve drought resistance in sugarcane breeding (Silva *et al*., 2008). Moreover, reduction in stomatal conductance (to reduce water loss) and an increase in density and deep root traits (to increase water uptake ability) have been reported as mechanisms for adaptation in sugarcane to maintain water status in the plant under water stress conditions (Wasson *et al*., 2012). Therefore, DS causes a decrease in stomatal conductivity to reduce water loss in the leaf. The resulting carbon dioxide entering through the stomata is also reduced as a result of a reduction in photosynthesis, which may also lead to low sugarcane yields. The mechanism of drought avoidance associated with root characters is to search for water in the soil layers. Under well-watered conditions, most sugarcane root systems remain in the upper soil layers (Smith *et al*., 2005), whereas a decrease in the moisture content of the soil surface activates growth of the roots in the lower soil

layers. The adapted root system acts to promote water absorption, thereby maintaining the water balance in the plant, and the adaptation of root system increases the amount of transpiration, which helps the sugarcane to avoid the danger of dehydration.

Studies of root traits and physiological parameters can be useful to select sugarcane genotypes for drought avoidance mechanisms. The standard method used in previous investigations is to evaluate root growth and distribution patterns of sugarcane under natural conditions provided by field experiments. However, among the methods for collecting root data, such as drawings, monoliths, and auger methods; considerable time, labor, and costs are involved and the actual growth of the sugarcane roots is not visible. Although a greenhouse experiment is an indirect and cost-effective means of studying root distribution, evaluation of plant root growth is limited to the early growth stage. Studies of root systems in the rhizobox are mostly done with young plants or annual plant species. The use of split-root systems to monitor the effect of root distributions on the development of the root system is very interesting (Neumann *et al*., 2009). The rhizobox is effective for displaying the characteristics of root distribution that can be shown on all root systems, and has been employed in many plant species such as peanuts (Thangthong *et al*., 2016, 2017, 2019) and Jerusalem artichokes (Puangbut *et al*., 2018), in which different varieties expressed differences in their root distribution patterns. However, the information on the changes in root distribution patterns under well-water conditions and drought conditions for characterization of sugarcane genotypes is still lacking.

The aim of this study was to investigate root distribution and physiological responses under both well-watered and drought stress conditions for various sugarcane varieties grown in rhizoboxes. The information obtained in this study is necessary for further experiments, and may be applied for the selection of sugarcane varieties for drought resistance.

MATERIALS AND METHODS

Plant material and rhizobox preparation

The experiment was conducted in rhizoboxes in a greenhouse at the Field Crops Research Station of Khon Kaen University, Khon Kaen, Thailand (16°28'N, 102°48'E, 200 m above sea level) from 21 June to 17 September, 2016. The experimental design consisted of a 2x13 factorial in completely randomized design, with two replications. Factor A consisted of two water regimes: well-watered and drought stress. Factor B contained 13 sugarcane genotypes: Yasawa, MPT03-320, PR3067, KK3, MPT06-166, K88-92, CP38-22, UT5, Kps01-12, KpK98-40, F152, BO14, and NCo382; which were selected by screening for differences in total root length at 21 and 35 days using a small pot experiment (Figure 1).

In this study, root distribution and root architecture were investigated using the modified box–pinboard method (needle-board) (Figure 2). The detailed method was clearly described in previous studies (Thangthong *et al*., 2016, 2017), and is briefly described herein. The
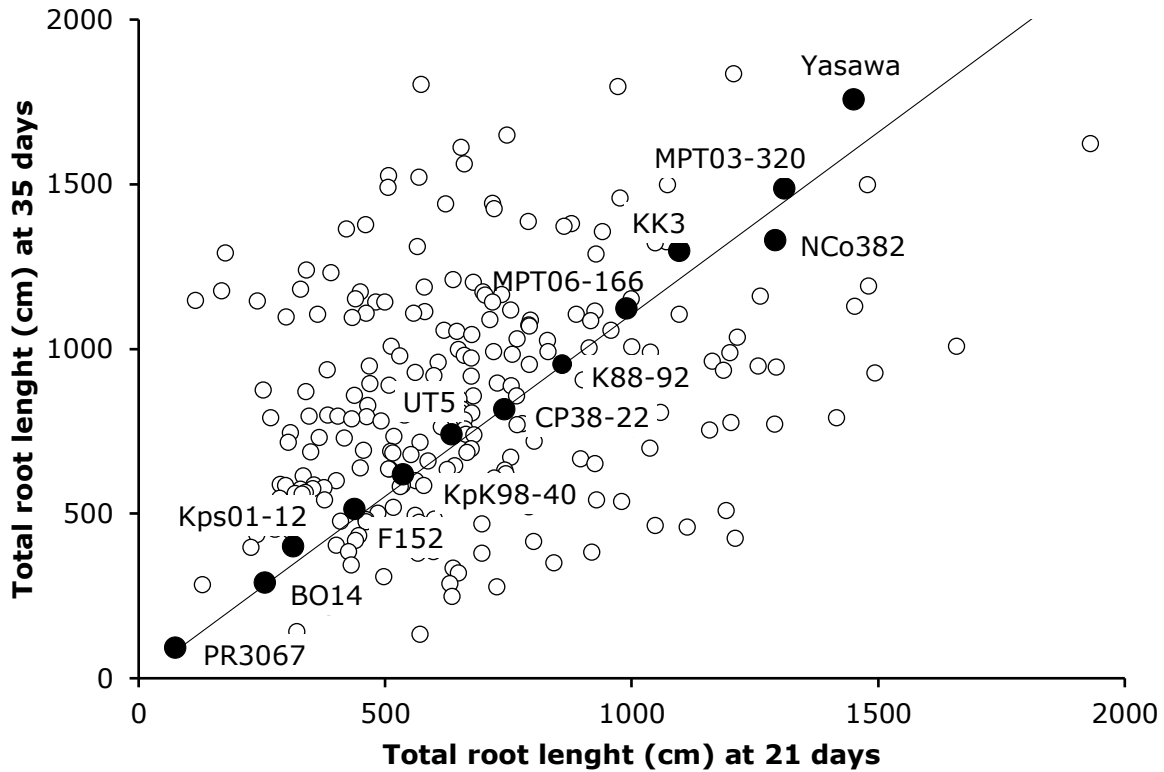
Figure 1. Relationships of 258 sugarcane genotypes in small pots. ○ = sugarcane genotypes, ● = 13 sugarcane genotypes were selected.
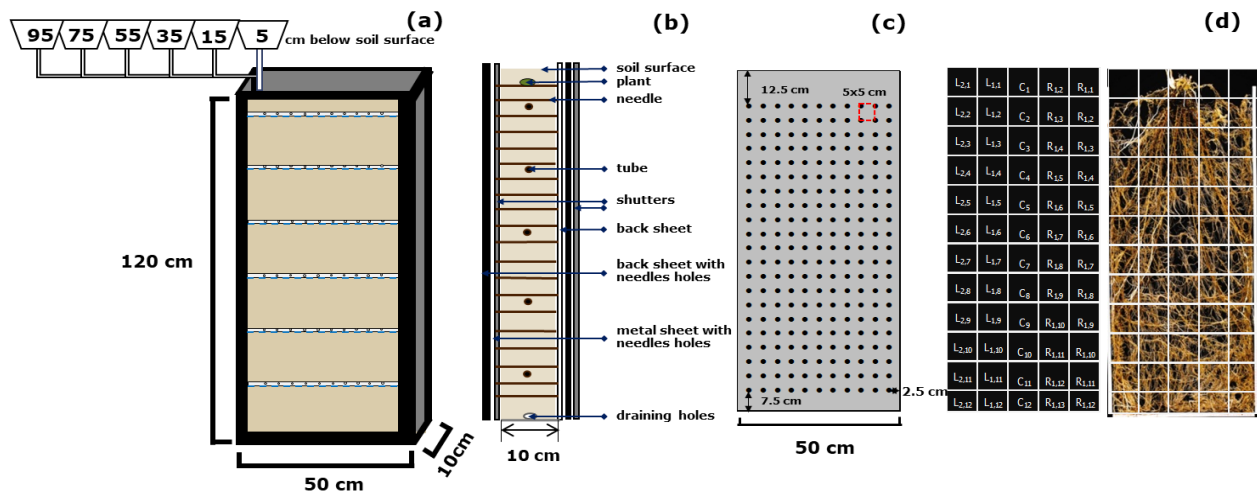

Figure 2. Diagrammatic representation and dimensions of each rhizobox, with the position of six tubes of irrigation the section showing different elements of the system (a), spacing of the needles at the backside of the rhizobox (b), the size of a square unit (10cm×10cm) of a square unit's observed area (c), and the image of sugarcane root system that was grown in a rhizobox at 90 days after planting (d).

rhizoboxes, 10 × 50 × 120 cm, were filled uniformly with dry soil to a height of 115 cm. The soil was then divided into 11 layers from the bottom of the boxes to the top of the boxes. The boxes had a needle grid at the back of the box, spaced 5 × 5 cm, in which to hold the roots in their original position after washing. A transparent window at the front of each box allowed for visually observation and photographs of root growth.

A sugarcane set with a germinated single bud was plated at the center of each box, 5cm below the soil surface. The boxes were wrapped with a black sheet on all sides, and then wrapped again with aluminum foil. The front side of the boxes could be easily opened, exposing the transparent window.

Irrigation treatments

Irrigated water was supplied to the rhizoboxes through a tube irrigation system. Six tubes were installed in each rhizobox at 5, 15, 35, 55, 75, and 95 cm below the soil's surface (Figure 2a); and, prior to transplanting, water was supplied at field capacity (FC) to all experimental units (rhizoboxes). At ten days after transplanting (DAT), water was provided at the soil surface of the two treatments, based on the water requirements of sugarcane for the uniformity of sugarcane set germination.

Two water regimes consisting of a well-watered (WW) level and drought stress (DS) level were created. At 10 DAT, WW treatment was supplied to the boxes through three upper tubes at 5, 15, and 35cm below the soil surface at FC level from initiation of the experiment until 45 DAT. At 45 DAT, WW treatment was supplied to the boxes at FC level until the end of the experiment through six tubes mounted at 5, 15, 35, 55, 75, and 95 cm below the soil surface.

DS treatment was supplied to the boxes at FC level from experiment initiation until 30 DAT through three tubes mounted at 5, 15, and 35 cm below the soil surface, and then the irrigated water was reduced to half of the FC level until 45 DAT. From 45 DAT to the end of the experiment, DS was supplied to the boxes at half of the FC level through three tubes mounted at 55, 75, and 95 cm at the lower soil layers. The soil moisture reduction was simulated similarly to that of typical field conditions in order to create higher soil moisture in the lower soil layers.

The water requirements of the sugarcane were calculated daily, as the sum of water loss through transpiration and soil evaporation based on the crop water requirements (ET_{crop}) (Doorenbos and Pruitt, 1992; Jangpromma *et al.*, 2012) as follows:

$$ET_{crop} = ET_o \times K_c$$

where ET_{crop} = crop water requirement (mm day^{-1}); ET_o = evapotranspiration of a reference plant under specified conditions, calculated by the Class A pan evaporation method (mm day^{-1}), and K_c = the crop water requirement coefficient for sugarcane.

Plant management

Before transplanting, each sugarcane genotype in each rhizobox was subjected to a curing process for five days until the buds and root primordial (0.5 cm) were germinated. Fertilizer grades 15-15-15 and 46-0-0 were applied at 1.56g per rhizobox at

1 DAT and 60 DAT, respectively. Soil moisture contents at FC (13%) and the permanent wilting points (4.3%) were determined via the pressure plate method.

Data collection

Soil moisture content

Soil moisture content was measured gravimetrically, using a micro-auger at 14, 28, 45, 60, and 90 DAT. Soil moisture content was collected at soil depths of 10cm (14 DAT); 10 and 25cm (28 and 45 DAT); 10, 25, 45, 65, and 85cm (60 DAT); and 10, 25, 45, 65, 85, and 105cm (90 DAT). Soil moisture content for each rhizobox was calculated as follows:

$$\text{Soil moisture content (\%)} = \left[\frac{(\text{wet weight} - \text{dry weight})}{\text{dry weight}}\right] \times 100$$

(2)

Physiological characteristics

SPAD chlorophyll meter reading (SCMR), chlorophyll fluorescence, stomatal conductance, and RWC were recorded in each rhizobox at 90 DAT. All characteristics were recorded from 09.00 am till noon. SCMR was recorded on the second or third fully-expanded leaf from the top of the main stalk using an SPAD-502 chlorophyll meter (Minolta SPAD-502 meter, Tokyo, Japan).

The same leaf samples were used for recording chlorophyll fluorescence using a chlorophyll fluorescence meter (MINI-PAM-2000, Heinz Walz GmbH, Germany). The leaf samples were stored under dark conditions for 30 minutes, and chlorophyll fluorescence was recorded using leaf clips (FL-DC, Opti-Science, Wetzlar, Germany) according to the method of Maxwell and Johnson, 2000; described previously, to quantify the level of drought-induced photo-inhibition.

Stomatal conductance was measured on intact leaves. The second or third fully-expanded leaf from the top of the main stalk was used for measurement of the trait using a porometer (model AP4, Delta-T Devices, Cambridge, UK).

The same leaf samples for measurement of stomatal conductance were used for the measurement of relative water content (RWC). The samples were harvested from the plants, and the leaf fresh weights were recorded. The leaf samples were cut into 3 pieces, width depending on leaf size of 13 sugarcane genotypes and length 3cm, placed in deionized water for 24 hours at room temperature, and leaf turgid weights were recorded. Leaf dry weight was measured after oven-drying at 80ºC for 48 hours (Silva *et al.*, 2007). RWC was calculated using the following formula:

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

(3)

Root and shoot dry weight

At 90 DAT, plant shoots were cut at the soil surface and shoot fresh weights were recorded. The samples were then oven-dried at 80ºC for 48 hours and the shoot dry weights were recorded. After scanning for root length measurement, the root samples were oven-dried at 80ºC for 48 hours, and the root dry weights were recorded.

Root characteristics

Root traits were measured at 90 DAT. The rhizoboxes were carefully washed with tap water to clean the root samples, and the needle grids were removed. Thereafter, two procedures were used to determine root traits: (i) photographic study, using a CanonEOS5D Mark IV24-70 f2.8 (Canon Ltd., Tokyo, Japan), and (ii) root scanning, via an Epson (Perfection V700, Japan) for analysis of the root lengths. The photographs showed the root distribution patterns of the whole root system on a black sheet with a scale bar (Figure 2d). Roots were separated into square sections taken from the left, center, and right columns. A root sample of each rhizobox was divided into 11 soil layers at 10cm intervals from the top to the bottom of the box.

In the root scanning procedure, the sample was separated into square unit sizes of 10cm×10cm (Figure 2d). Root length was analyzed by the WinRHIZO program (WinRHIZO Pro(s) V.2004a, Regent Instruments Inc. Canada) for the root distribution patterns. Root lengths in the upper soil layers of 0–10, 10–20, and 20–30cm were combined into a single 0–30cm layer; whereas root traits at the lower soil layers were combined to form a single layer of 30–110 cm.

Statistical analysis

Analysis of variance was performed for each characteristic following a factorial in completely randomized design, and the data for each water regime was analyzed separately. The least significant difference (LSD) was used to compare means. All analyses were performed using the Statistix 10$^{©}$ software program.

RESULTS

Soil moisture content

As expected, soil moisture content were different for sugarcane varieties grown under well watered and drought stress conditions from 45 to 90 DAT (Figure 3a). In the soil depths of 10–85cm at 60 DAT (Figure 3b) and 10–105cm at 90 DAT (Figure 3c) soil moisture content were also different between water regimes.

Root distribution pattern of sugarcane

Images of the root distribution patterns of all 13 sugarcane genotypes grown in the rhizoboxes and captured at 90 DAT revealed the root distribution patterns of all the genotypes under WW and DS conditions (Figure 4). Superficial roots (roots emerging from higher nodes) were thinner and highly branched, extending laterally to form a dense network of responsible for uptake of water and nutrients from surface soil layers (Evans, 1935) were observed to appear mostly in the upper soil layers (Figures 4 and 5).

Root length of the sugarcane genotypes were reduced in the upper soil layers (0–30cm) in response to drought stress. However, the roots in the lower soil layers (below 30cm) increased. KK3, K88-92, CP38-22, KpK98-40, BO14, and NCo382 produced new roots (white in color) within the lower soil layers, whereas PR3067 and F152 increased buttress roots in the lower soil layers, in order to take up water and minerals from deep soil layers, especially under drought stress (Figure 4).

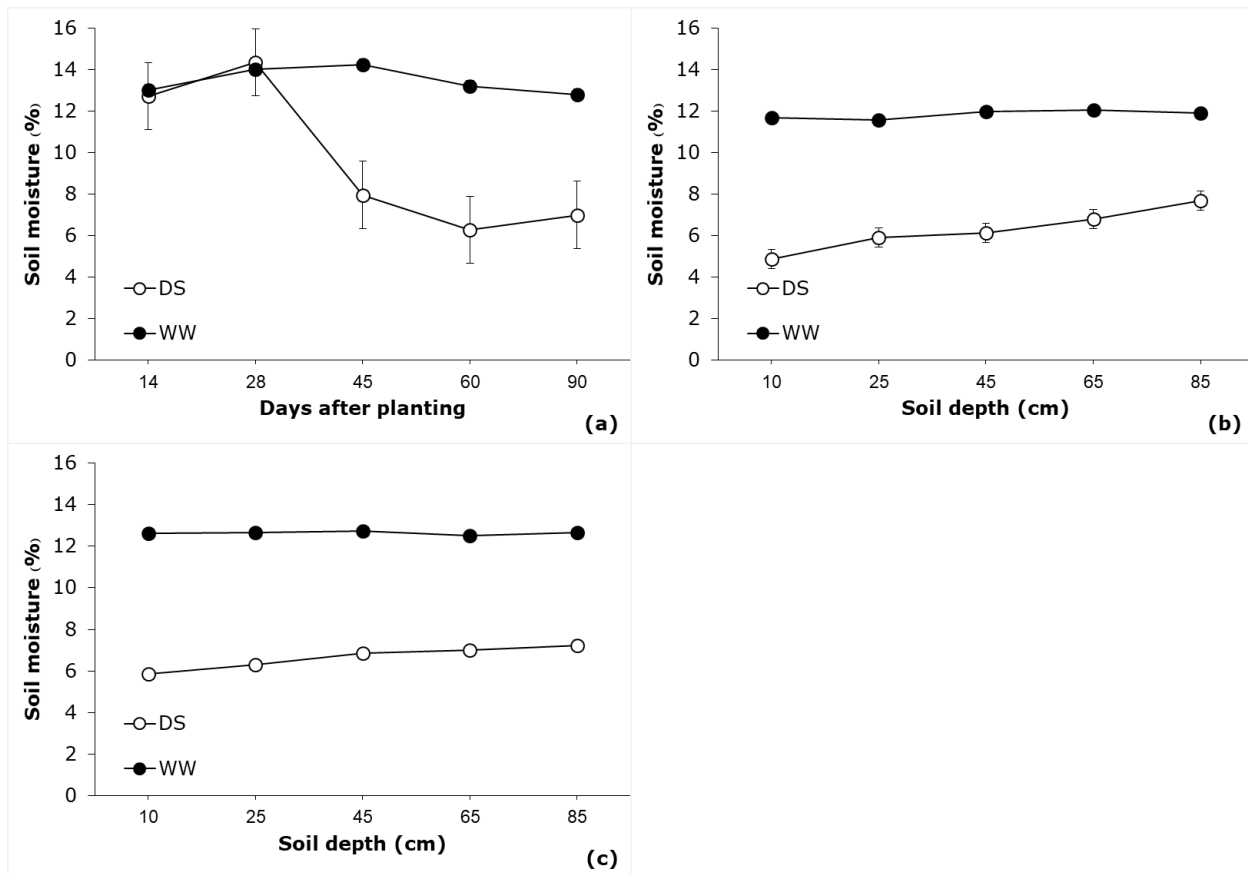KK3, K88-92, CP38-22, Kps01-12, and BO14 increased buttress roots

Figure 3. Soil moisture content (%) of drought stress (DS) and well-watered (WW) treatments at 14, 28, 45, 60 and 90 DAP (a), in different soil layers 10, 25, 45, 65 and 85 cm at 60 DAP (b) and 90 DAP (c). The bar is standard error (SE) for difference between two means statistically significant (*P* ≤ 0.01)

within the lower soil layers (Figures 4 and 5). Under well-watered conditions, K88-92, KpK98-40, BO14, and NCo382 developed superficial roots on the soil surface (Figure 4); and CP38-22 and KpK98-40 maintained high root growth as indicated by their extended root length.

KK3 had higher root length in lower soil layers under drought stress condition than under well-watered conditions (Figures 4 and 5). Kps01-12 under drought stress was capable of maintaining high root lengths in both upper and lower soil layers. K88-92 and BO14, under well-watered conditions, developed high root

lengths in both upper and lower soil layers. Under drought stress conditions, these genotypes reduced root length in upper soil layers, and increased root lengths in lower soil layers. Under drought stress conditions, MPT03-320 and MPT06-166 reduced root lengths in the upper soil layers to maintain root growth in the lower soil layers.

The DS and WW treatments were compared for root length and root distribution at 90 DAT (Figure 6). For each of the 11 soil layers (at 10 cm intervals from the top to the bottom of the rhizobox), the root length increased at the initiation stage

Figure 4. Root distribution patterns of 13 sugarcane genotypes grown in rhizoboxes under drought stress (DS) and well-watered (WW) conditions, at 90 days after

Figure 5. Graphical presentation describing root length distribution patterns of 13 sugarcane genotypes grown in rhizoboxes under drought stress (DS) and well-watered (WW) conditions, at 90 days after planting.
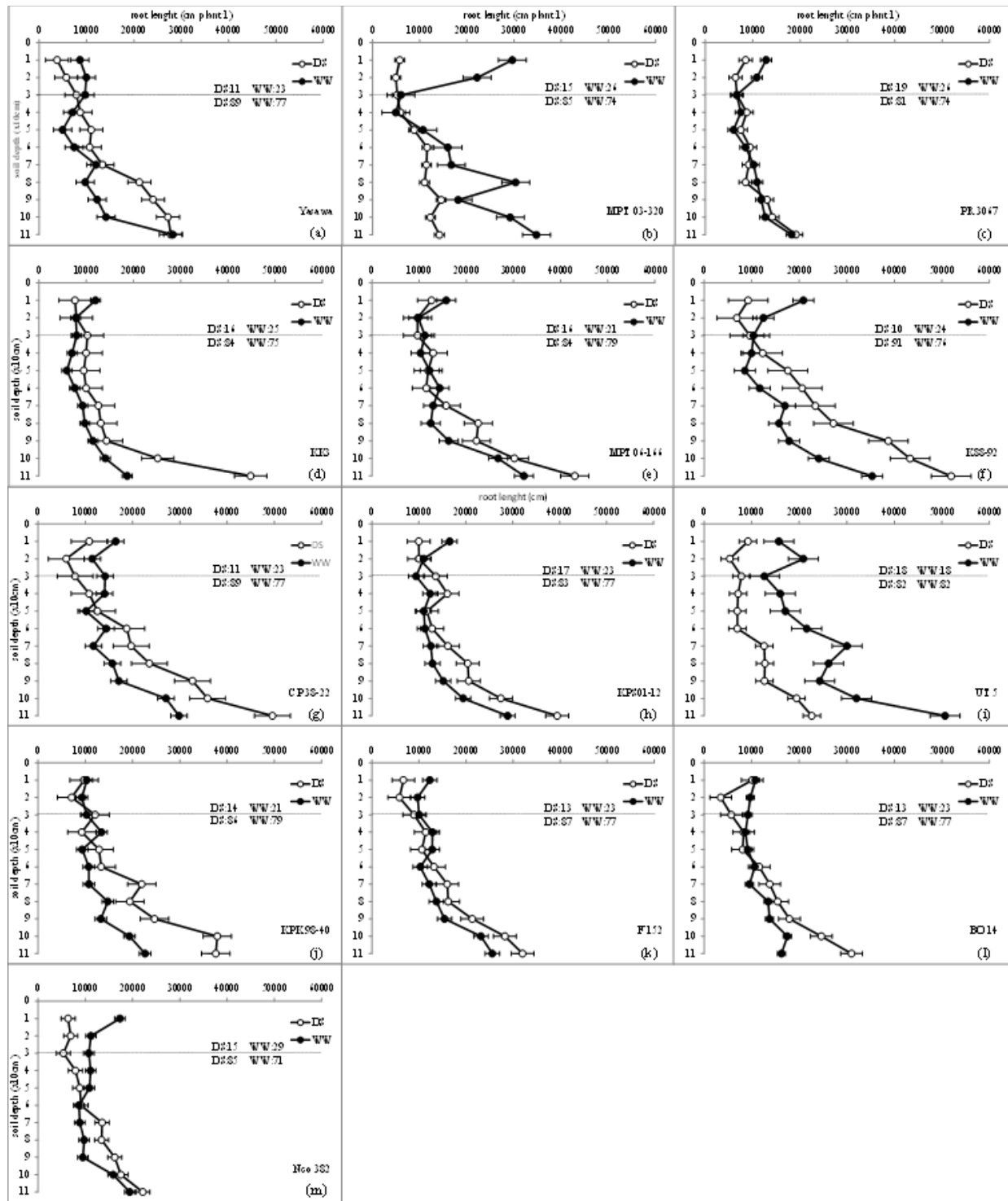
Figure 6. Root length distribution of sugarcane under drought stress (DS) and well-watered (WW) of 11 (10cm interval) layers, and separated by percentage for two soil layers defined previously as upper (0–30cm of soil depth) and lower (30–120 cm of soil depth), evaluated at 90 days after planting. The bar is standard error (SE) for difference between two means statistically significant ($P \leq 0.01$).

up to 90 DAT. The sugarcane crops grown under drought treatments and well-watered treatments were significantly different ($P \leq 0.01$) for root length at 90 DAT. The percentage of root length in the upper soil (0–30cm) was lower than that in the lower soil (below 30cm) (Figure 6). Root lengths of most sugarcane genotypes grown under DS conditions were longer than those under WW conditions in most of the soil layers (Figure 6), except for MPT03-320, PR3067, and UT5 genotypes; which demonstrated an opposite trend (Figure 6). All sugarcane genotypes grown under drought conditions had higher percentages of root length than did the genotypes grown under well-watered conditions. Most sugarcane genotypes grown under drought conditions had lower root percentages in the upper soil layers than did these same genotypes grown under well-watered conditions, except for UT5, which had a similar percentage of root length under both drought and well-watered conditions (Figure 6). However, the patterns of root growth between DS and WW treatments were rather different in all 13 genotypes, as the sugarcane genotypes grown under drought conditions produced higher percentages of roots and root lengths in lower soil layers than did these genotypes grown under well-watered conditions.

Responses of root characteristics to water regimes

Sugarcane genotypes were significantly different ($P \leq 0.01$) in total root length. Drought stress increased total root length in KK3, MPT06-166, K88-92, CP38-22, Kps01-12, and KpK98-40. It reduced root lengths in MPT03-320 and UT5, whereas Yasawa, PR3067, F152, BO14, and NCo382 were not significantly affected by drought stress (Figure 7a).

Under drought conditions, KK3 and Kps01-12 maintained root dry weight (root size), similar to those under well-watered conditions, however, root length was increased. MPT06-166, K88-92, CP38-22, KpK98-40, and BO14 decreased root dry weight, while also increasing root length. MPT03-320, UT5, and NCo382 displayed a decrease in both root dry weight and root length. For the remaining genotypes, the results were not significantly different between the two water regimes (Figure 7b).

MPT03-320, KK3, MPT06-166, K88-92, CP38-22, Kps01-12, UT5, KpK98-40, BO14, and NCo382 had an increased root length-to-root dry weight ratio under DS conditions. The opposite trend was observed for Yasawa, PR3067, and F152 (Figure 7c).

Total dry weight, shoot dry weight, and root-to-shoot ratio

Sugarcane genotypes were significantly different ($P \leq 0.01$) for total dry weight, shoot dry weight, and root-to-shoot ratio under both drought stress conditions and well-watered conditions (Figures 8a–c). Under well-watered conditions (Figure 8a); MPT03-320, K88-92, CP38-22, UT5, BO14, and NCo382 had high total dry weight; KpK98-40 had a moderate total dry weight; and Yasawa, PR3067, KK3, MPT06-166, Kps01-12, and F152 had low total dry weight. Under drought stress conditions, MPT03-320, K88-92, CP38-22, UT5, and NCo382 reduced total dry weight; PR3067 increased total dry weight; and Yasawa, KK3, MPT06-
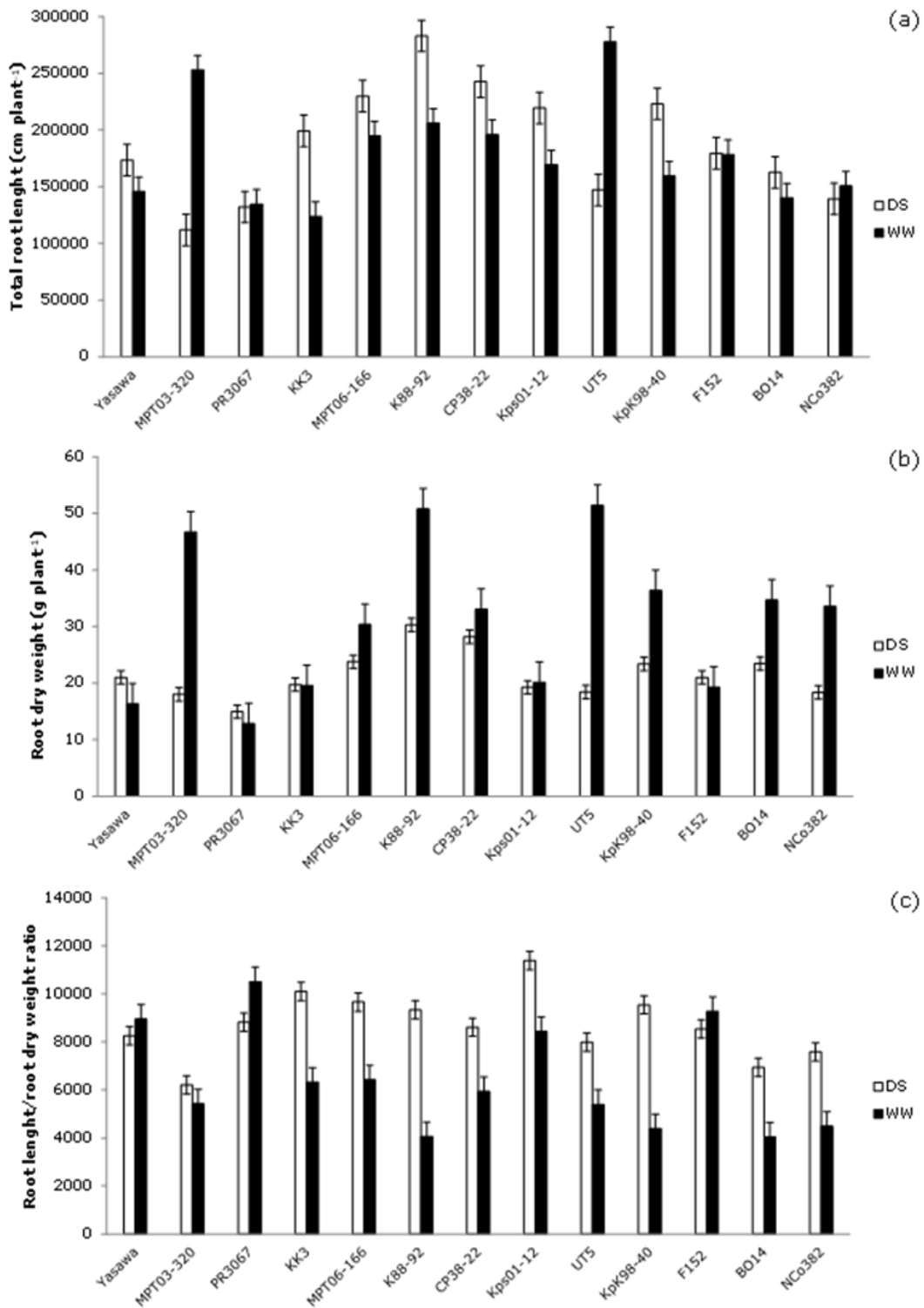
Figure 7. Total root length (a), root dry weight (b) and root length/root dry weight ratio (c) of 13 sugarcane genotypes grown in a rhizobox under drought stress (DS) and well-watered (WW) conditions. The bar is standard error (SE) for difference between two means statistically significant ($P \leq 0.01$)
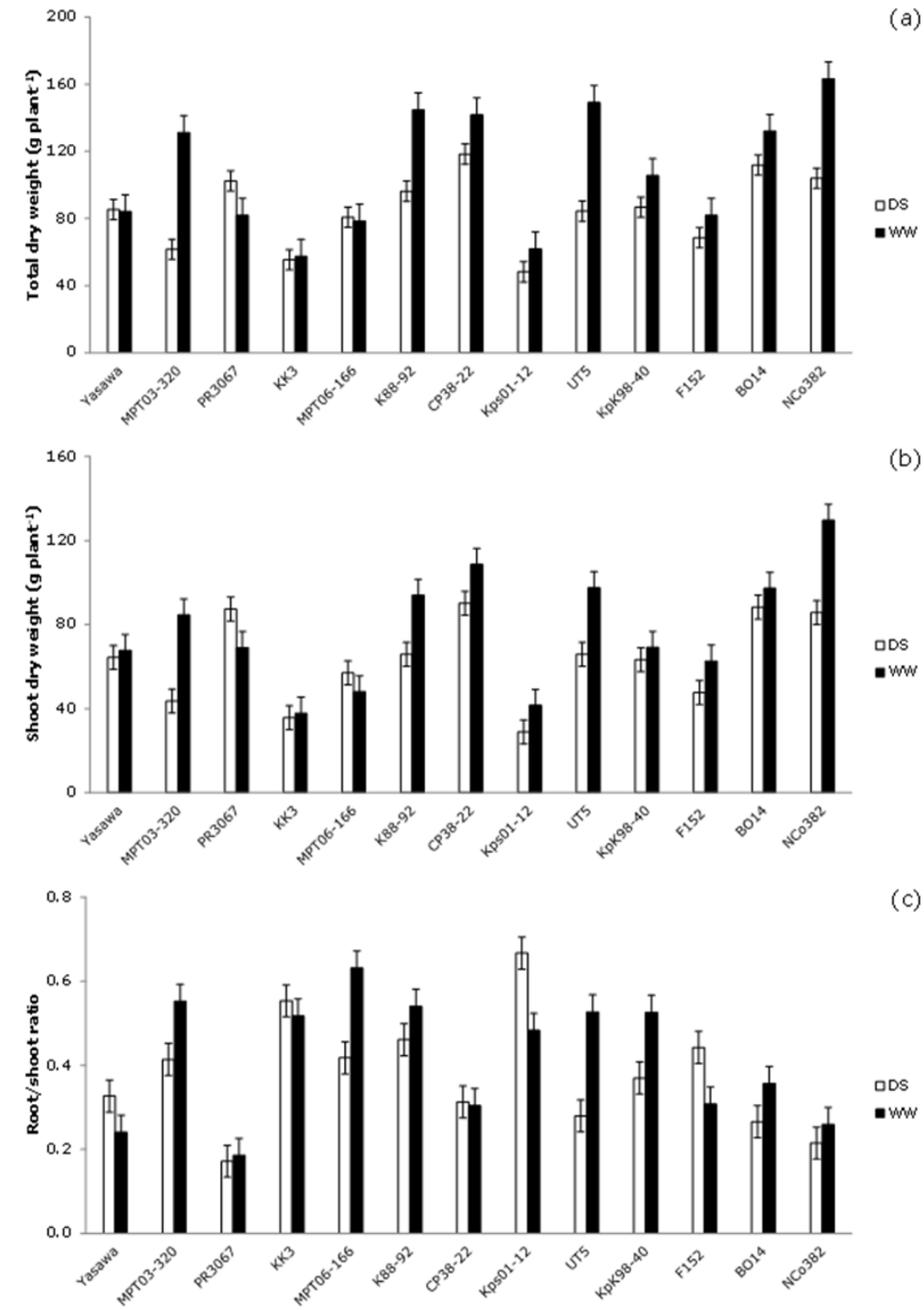
Figure 8. Total dry weight (a), shoot dry weight (b) and root/shoot ratio (c) of 13 sugarcane genotypes grown in rhizoboxes under drought stress (DS) and well-watered (WW) conditions. The bar is standard error (SE) for difference between two means statistically significant ($P \leq 0.01$).

166, Kps01-12, KpK98-40, F152, and BO14 had similar total dry weight under both drought stress and well-watered conditions.

MPT03-320, K88-92, CP38-22, Kps01-12, UT5, and NCo382 reduced shoot dry weight; PR3067 increased shoot weight; and Yasawa, KK3, MPT06-166, KpK98-40, F152, and BO14 had similar shoot dry weights under both drought stress and well-watered conditions (Figure 8b). Total dry weight and shoot dry weight, under drought stress conditions, MPT03-320, MPT06-166, K88-92, UT5, KpK98-40, and BO14 reduced root-to-shoot ratios. Yasawa, Kps01-12, and F152 increased root-to-shoot ratio; whereas PR3067, KK3, CP38-22, and NCo382 had similar root-to-shoot ratio under both drought stress and well-watered conditions (Figure 8c).

Relationships of root traits and above-ground traits within stress and non-stress environments

Sugarcane varieties grown under drought stress conditions and well-watered conditions the correlations were positively significant ($P \leq 0.05$ and 0.01) for shoot dry weight, total dry weight, and root-to-shoot ratio (r = 0.74, 0.63, and 0.57, respectively) (Figures 9c, 9d, 9f). However, the correlations between drought conditions and well-watered conditions of sugarcane varieties were not significant for total root length, root dry weight, and root length-to-root dry weight ratio (Figures 9a, 9b, 9e).

K88-92, CP38-22, and MPT06-166 had high root lengths under well-watered conditions (high potential) as well as under both well-watered and drought stress conditions. KpK98-40, Kps01-12, KK3, and F152 increased root length under drought stress

conditions; whereas MPT03-320 and UT5 reduced root length under drought stress conditions. Yasawa, BO14, NCo382, and PR3067 maintained similar root lengths under both drought stress and well-watered conditions.

Relationships between root and physiological traits under stress and non-stress conditions

The correlations between stomatal conductance and total root length were positively significant under both well-watered conditions (r = 0.81, $P \leq$ 0.01) and drought stress conditions (r = 0.75, $P \leq$ 0.01) (Figures 10c and 11c). The correlations between the other physiological traits and root traits were not significant. Under drought stress and well-watered conditions, K88-92, CP38-22, and MPT06-166, which maintained high root lengths, were strongly associated with high stomatal conductance (Figures 10c and 11c).

Relative water content indicates the plant's water status. The sugarcane genotypes grown under drought stress conditions and under well water conditions at 90 DAT were significantly different for relative water content (Figures 10b, 11b). Under drought stress conditions, K88-92, CP38-22, and MPT06-166 had low relative water contents (Figure 10b). The differences in relative water content between drought stressed crops and well watered crops would be due to differences in soil moisture content. Relative water content was appropriate for the evaluation of plant water status, as this trait associated with soil moisture content.

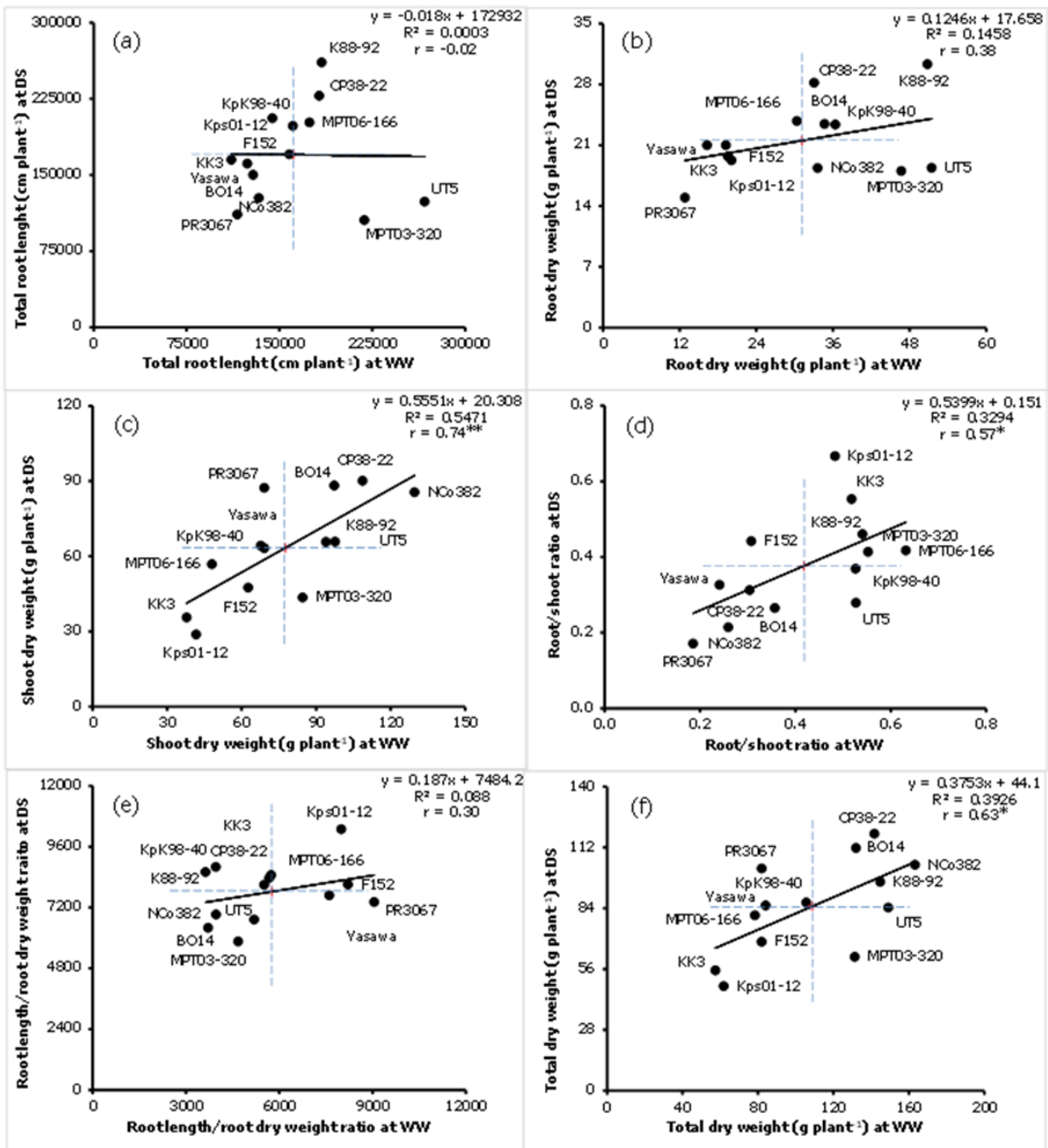For the genotypes K88-92, MPT03-320, CP38-22, MPT06-166, and UT5, presenting high total root

Figure 9. Relationships between drought stress (DS) and well-watered (WW) conditions of root length (a), root dry weight (b), shoot dry weight (c), root/shoot ratio (d), root length/root dry weight ratio (e), and total dry weight (f).
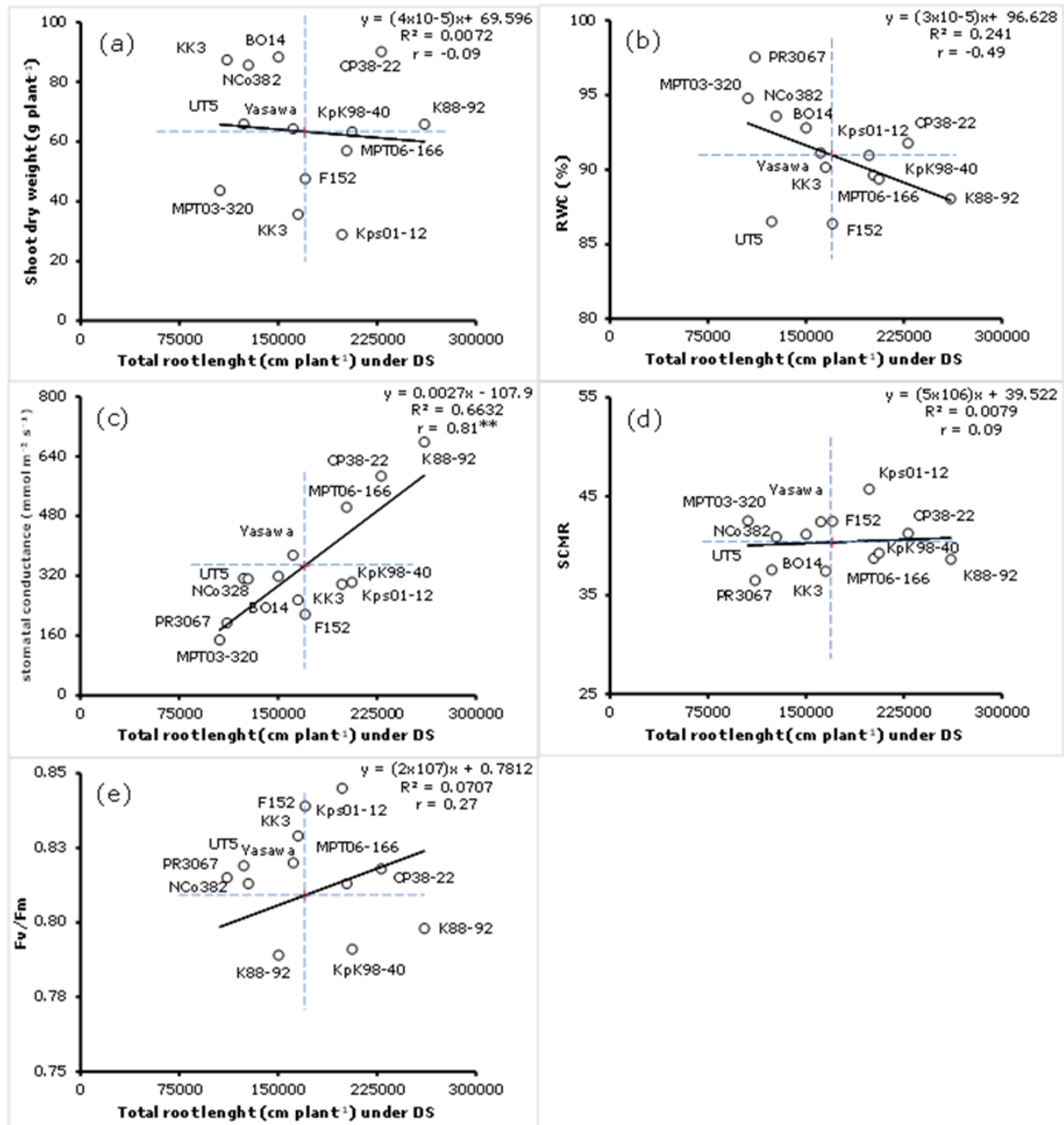
Figure 10. Relationships between root length and shoot dry weight (a), relative water content (RWC) (b), stomatal conductance (c), SPAD chlorophyll meter reading (SCMR) (d) and chlorophyll fluorescence (e), under drought stress (DS) conditions.
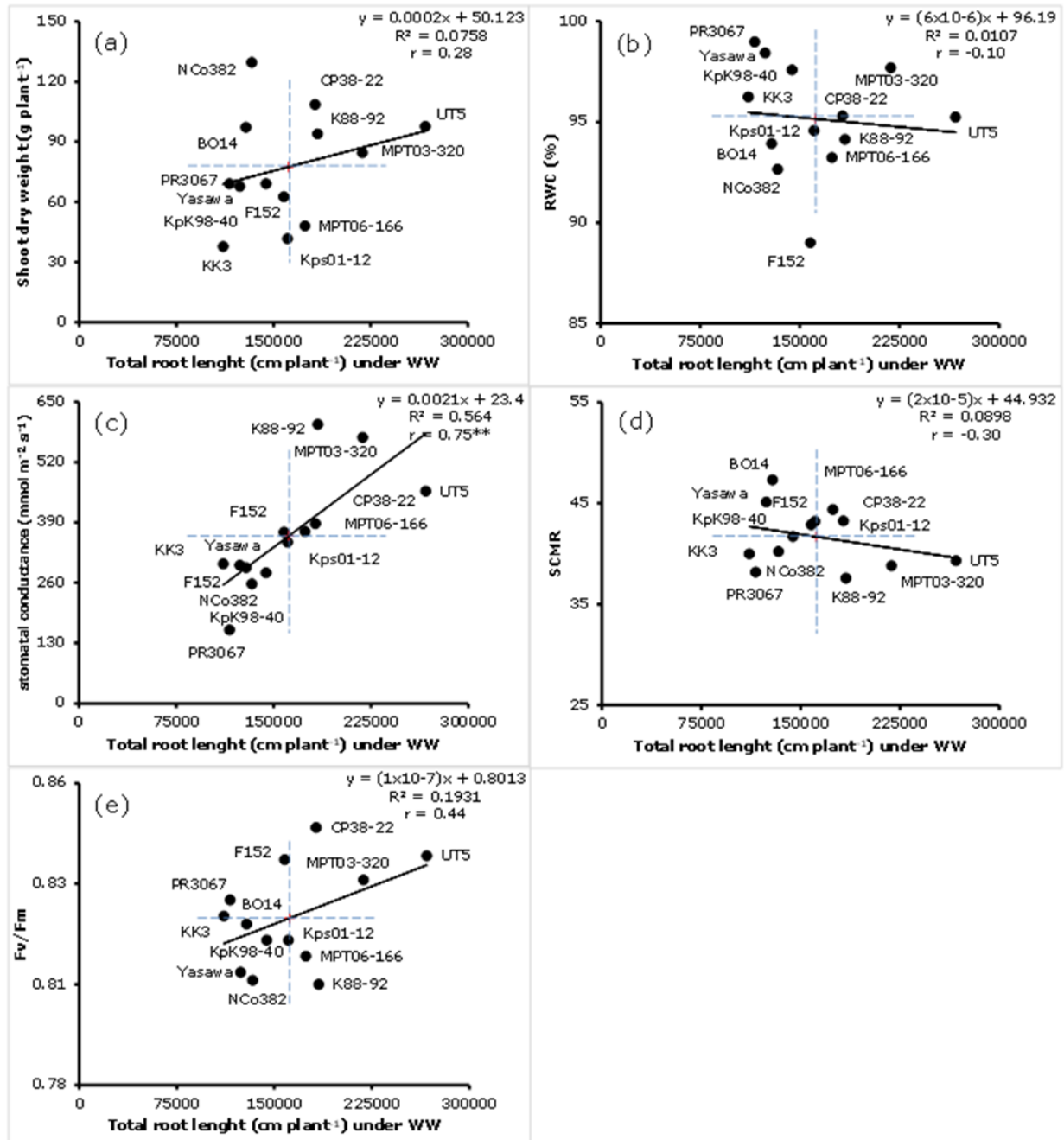
Figure 11. Relationship between root length and shoot dry weight (a), relative water content (RWC) (b), stomatal conductance (c), SPAD chlorophyll meter reading (SCMR) (d), and chlorophyll fluorescence (e) under well-watered (WW) conditions.

lengths per plant under well-watered conditions; total root length was associated with stomatal conductance (Figure 11c), whereas total root length was not associated with relative water content (Figure 11b). Sugarcane genotypes were significantly different for total root length under drought stress conditions. The correlation coefficients between root length and shoot dry weight (Figures 10a and 11a), RWC (Figures 10b and 11b), SCMR (Figures 10d and 11d), and chlorophyll fluorescence (Figures 10e and 11e) were not significant.

DISCUSSION

Root development for sugarcane growth

Root growth and distribution under diverse environments are important for predicting the responses of a plant to changes in the soil and environment. Drought tolerance strategies regularly include the development of expanded or deep root structures and other physiological functions, such as oxidative stress protection and a decrease in transpiration and osmo-regulation (Pirnajmedin *et al*., 2015). The main finding of this study is that water-limited conditions change the root distribution patterns of the different sugarcane genotypes investigated. Plant roots grew into deeper soil layers in response to a decrease in soil water. The differential responses of sugarcane genotypes to drought stress for root traits and other physiological traits may be dependent on genetic control. Under water-limited conditions, the morphology of the root structure is essential in accessing nutrients and soil water (Smith *et al*., 2005). Rapid development and suitable distribution of the sugarcane root system at deeper soil layers are essential to curtailing the adverse consequences of these dry periods on yield. Under well-watered conditions, root density was high and uniform throughout each soil layer (Kato and Okami, 2011). Under water-limited conditions, a plant invests more in root growth than in shoot growth, in order to take up more water. Under water-deficit conditions, the translocation of assimilates to roots was higher than to shoots (Azhiri-Sigari *et al*., 2000).

In the course of the early growth stages of the water-limited conditions, progressive accumulation of root dry matter was at the expense of shoot growth, where plants capable of adapting to dry conditions produced higher root/shoot ratios. Once a decrease in the soil moisture content is detected, roots must expand their distribution patterns and elongate into deeper soil layers for extracting and engaging a larger soil volume for water. As soil moisture at the soil surface and in the top soil profile was diminished under water deficit stress, the roots removed more water at a deeper profile. A deep root scheme is helpful for extracting water form substantial soil depths (Kavar *et al*., 2007). This root system characteristic is an important consequence to soil drying and allows some roots to continue elongation under a water deficit to search more water. The distribution of the root schemes depends strongly on the soil moisture of the deeper soil layer.

Root characteristics under water deficit conditions

Water deficits may adversely affect root growth in the upper soil layers, as they are dryer than the lower soil layers. Root growth in upper soil layers is then limited by drying soil, whereas root growth in the lower soil layers is continues in response to soil moisture. Root response to soil moisture in the lower soil is an important characteristic necessary to enhance water extraction from deeper soil, and improve the plant's potential to resume growth during drought. It has been proposed that breeding for a narrow xylem vessel in the seminal roots of wheat should accrue the hydraulic axial resistance, and enforce plants to apply the subsoil water more slowly (Passioura, 1972). Under drought stress conditions, several root morphological traits are modified in response to drought, in which the morphological modification affects total root length. Small diameter roots enable plants to efficiently enhance hydraulic conductance by increasing the surface area in contact with soil water and the soil volume that can store more water, and also help increase the roots' hydraulic conductivity by decreasing the apoplastic barrier of water entering the xylem (Comas *et al.*, 2012; Hernández *et al.*, 2010). Consequently, a decrease in root diameter has been suggested as a trait for increasing the plant's ability to hold water and improve productivity under water deficit conditions (Wasson *et al.*, 2012).

Under drought conditions, roots below 55 cm grow rapidly into lower soil layers in order to extract soil water from moist soil. The change in root growth in response to drought may indicate drought resistance under water deficit conditions. Under drought conditions, root lengths at soil layers of 55, 75, and 95 cm changed the distribution patterns compared to root lengths under well-irrigated conditions. Root biomass in the deeper soil layers consisted of newly growing roots, elongating roots from old primary roots in the soil layers, and branching roots from the roots already in existence (Azhiri-Sigari *et al.*, 2000). Under drought conditions, the lateral roots were induced by drying soil in the upper soil layers to develop new roots that grow into moist soil found in the lower soil layers (Nagel *et al.*, 2015). The distribution and architecture of the root structures may depend strongly on the moisture of the deeper soil layers. In this experiment, differences in irrigated water caused drastic water deficits in drought treatment and changed the root distribution patterns of sugarcane. Under water stress conditions, the root lengths of sugarcane in the lower soil layers were longer than under well-watered conditions, and root growth reduces the food that is supplied to shoots.

Within an early season drought, roots below 55cm of soil have more root tips (root apex zones) than those in the upper soil layers. Soil moisture in the lower soil layers was higher than the permanent wilting point, as water was obtainable for the plants. Water stress changed the root structure patterns and increased the root length of the roots below 55cm. Water deficits increase the root length and the percentage of roots in the lower soil layers (Songsri *et al.*, 2008; Jongrungklang *et al.*, 2011). In many of the aforementioned studies, water stress increased elongation of roots in deep soil layers, through both the core

sampling and auger methods, in which the recovery of whole root systems was not necessary to illustrate the complete root distribution pattern. This behavior suggests that root responses at given periods of water stress were determined essentially by root length and a change in the distribution pattern.

The root system may prove to be a more important sink than the upper portion of the plant under water deficit at the vegetative stage. The effects of root percentage and root size may be indicative of a drought resistance mechanism under water-limited conditions. The positive relationship between root length and soil water content by the end of the drought period below the 40cm soil layer (Figure 6) shows the advantage of an increase in deep roots, needed to extract water from deep soil layers over extended periods. Nonetheless, the association between root length and physiological responses to plant water status are very complicated. Several root systems are considered to be essential in sustaining plant productivity under a water deficit. The overall size of a root system is related to the acquisition of water and nutrients from the soil, and can be associated with drought resistance and yield performance under drought.

Physiological traits under different water regimes

Shifts in allometry (metrics of root-to-shoot relationships) and shoot stature can compensate for water shortages; and along with shifts in stand densities, can maintain stomatal conductance under xeric conditions (Mencuccini, 2003; Maseda and Fernandez, 2006). Root lengths in deeper soil is an important trait for

preserving stomatal conductance under water-limited conditions. The rationale for imposing boundary conditions at the stem base is that water fluctuation through plants is primarily controlled by stomatal conductance. Indeed, the stomatal conductance is dependent upon both soil moisture and relative water contents. Under water deficit conditions, stomatal closure usually occurs in the afternoon, which can occur even under WW conditions under high evaporative demand. The stomata closure generally occurs earlier in the day, as the soil water reserve is depleted, so stomatal closure in the early morning occurs only with a very low soil water potential (Tardieu *et al.*, 1992). Therefore, the soil water potential value that stops water uptake can be interpreted as triggering the stomatal closure and transpiration arrest, even in the early morning.

Studies of plants exposed to drought stress conditions focus on traits, such as root architecture and physiological features (i.e., leaf water potential, osmotic adjustment, and RWC) at the vegetative stage (Basu *et al.*, 2016). The C_4 plants grown under well-watered conditions with elevated CO_2 levels reduced stomatal conductance that can lead to enhanced leaf growth and photosynthesis by mitigating the effects of transient water stress (Seneweera *et al.*, 1998). Root systems of plants responded to soil fertility and soil moisture. Root growth was affected primarily by increased RWC (40–51%) (Derner *et al.*, 2001). Drought-tolerant cultivars of sugarcane maintain a high RWC (Boutraa *et al.*, 2010). Longer roots in the lower soil layers, in response to drought, are important for plant

resilience (Songsri *et al.*, 2008; Jongrungklang *et al.*, 2013). The selection of genotypes with high root lengths in lower soil layers will likely improve stomatal conductance and enhance photosynthetic capacity and plant growth within a drought-prone environment.

CONCLUSION

The sugarcane genotypes displayed different root distribution patterns and architectures. The adaptation of sugarcane subjected to DS conditions included a reduction in root lengths in the upper soil layer, and increased root lengths in deeper soil. Differences in the adaptation of the sugarcane genotypes were found for root traits under drought stress, with KK3, MPT06-166, K88-92, CP38-22, Kps01-12, and KpK98-40 demonstrating high root lengths in the deeper soil, which further demonstrates a trait which may be identified as a drought avoidance mechanism. The present study further revealed that enhanced root length in deeper soil layers is an important trait for maintaining stomatal conductance under drought conditions, and a useful trait for parental selection in future breeding programs, again, as a drought avoidance mechanism.

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