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ABSTRACT

Water deficit stress is a major factor that inhibits the overall growth and development in cassava (*Manihot esculenta*), leading to decreased storage root yield. We conducted a study to investigate whether thermal sensing could be used to indicate water deficit stress and the health and yield of cassava crops in field. The objective of the study was to use thermal imaging to determine relationship between crop water stress index (CWSI) and physiological changes, and to identify the critical CWSI point in fields of cassava cv. Rayong 9 under well-irrigated and water-deficit conditions. At the time of storage root initiation (85 DAP [day after planting]), thermal imagery was collected and the physiological changes and growth characters were measured prior to storage root harvesting (162 DAP). Thermal infrared imager was used to measure the canopy temperature and CWSI of cassava plants. Net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rates (T_r) of cassava plants under water deficit conditions for 29 d (114 DAP) were significantly decreased, leading to delayed plant growth as compared to those under well-irrigated conditions. In contrast, air vapor pressure deficit (VPD_{air}) and CWSI in drought-stressed plants were higher than well irrigated plants. High correlations between $T_r/g_s/P_n$ and CWSI were observed. The study concludes that CWSI is a sensitive indicator of water deficit stress caused due to stomatal function.

Abbreviations: CWSI: crop water stress index; DAP: day after planting; Pn: net photosynthetic rate; gs: stomatal conductance; Tr: transpiration rate; VPDair: air vapor pressure; RMSE: root mean square error

1. Introduction

Cassava (*Manihot esculenta* Crantz; Euphorbiaceae), a perennial crop, is one of the most important staple foods in the tropical countries and considered as the fourth most important source of energy (Balagopalan, 2002). In Asia, cassava is demanded for food and many industrial uses such as for the production of ethanol, animal feed, etc. (Parmar, Sturm, & Hensel, 2017). Cassava is commonly grown on upland areas, receiving less than 800 mm annual rainfall with a dry period of 4–6 months which is important for flour storage. Although it is classified as a drought tolerant crop, the growth characters and yield performances decline under prolonged dry periods (El-Sharkawy, 2004). Reduction in the storage root yield depends on the duration of the water deficit conditions and the growth stage of the storage root (El-Sharkawy, 2006). The critical period of cassava cultivation that could be affected by water deficit conditions ranges from 1 to 5 months

after plantation (MAP), identified as the root initiation and tuberization stages (El-Sharkawy, 2007). Continuous water deficit for at least 2 months during this period can reduce storage root yield by 32–60% (Hillocks, Thresh, & Bellotti, 2002). Soil moisture stress in cassava field led to decline in plant height, stem girth, number of tubers and tuber yield by 47, 15, 95 and 87%, respectively (Aina, Dixon, & Akinrinde, 2007). In response to prolonged water stress, reduced shoot biomass and storage root were observed in cassava with significant reduction during early water stress period (2–6 MAP) (El-Sharkawy, 2012). The primary response to water stress is stomatal closure. The stomata rapidly close as the leaf water potential decreases, the vapor pressure deficiency increases and both rate of photosynthesis and transpiration decrease (Hillocks et al., 2002).

Thermal sensing (spectral range 7.5–14 μm long-wave infrared) is particularly useful for the study of plant-water relations and drought stress responses because of the key role of stomatal closure (Tanriverdi et al., 2017; Zaman-

Allah, Jenkinson, & Vadez, 2011). Canopy temperature is linearly related to the rate of water loss, which in turn is closely related to the stomatal conductance. The major problem with canopy temperature is that the measurement of stomatal conductance is very sensitive to the environmental factors. Precise monitoring of temperature under field conditions is hindered by several factors, for example, incident radiation, wind speed, vapor pressure deficit, soil moisture and microclimate around the canopy (Mishra et al., 2016; Walter et al., 2010). Thermal sensing, however, is a simple, rapid, repeatable, cost effective, highly sensitive and a nondestructive method and it can further be validated with remote sensing technology, which are appropriated over stomatal conductance measurement using Portable Photosynthesis System (Pask et al., 2011). Therefore, it is necessary to normalize the observed canopy temperature to get a useful general indication of stress. The development of water stress indices can normalize the canopy temperature against air temperature. In these indices, a stress degree day is defined as the difference between canopy temperature and air temperature at a specified time. Successive daily leaf temperature obtained near midday could be integrated over time to give an accurate measurement of crop stress (Blum, Mayer, & Gozlan, 1982; Hashimoto et al., 1984; Jones & Vaughan, 2010). The crop water stress index (CWSI) provides a mean of how the crop is responding to existing soil moisture conditions (Idso et al., 1981). In principal, nondestructive physiological responses, CWSI and stomatal conductance (g_s) in crop species under severe stress conditions have been well established as high correlation and may be calculated as effective indices by thermal imaging data (Rud et al., 2014; Xu et al., 2016). The CWSI is better determined by crop water status rather than the stress degree day method. The CWSI value is a measurement of the reduction in transpiration, expressed in decimals with values ranging from 0 (no stress) to 1 (maximum stress). CWSI depends on the plant species, developmental stage of the plant, extent of the water deficit stress and their interactions (Bijanzadeh & Emam, 2012; Blum et al., 1982; Wiriya-Alongkorn et al., 2013; Xu et al., 2016). A critical wilting point induced by a little amount of stress is acceptable, indicating the necessity of irrigation to recover the plants without yield loss. Consequently, stomatal closure can be detected through thermal sensing using CWSI and it has helped in scheduling the irrigation for many crop species (Xu et al., 2016). In previous studies, CWSI and physiological changes in rice (Xu et al., 2016), wheat (Alderfasi and Nielsen, 2001), corn (Irmak, Haman, & Bastug, 2000) and cotton (Choudhury, 1986) crops in response to water deficit stress have been well established. However in cassava, there is still lack of information on the CWSI and physiological responses at different developmental stages under water deficit stress. The main focus of this investigation was to use thermal imaging in order to determine relationship between CWSI and physiological changes, and to identify the critical CWSI point in fields of cassava cv. Rayong 9 under well-irrigated and water-deficit conditions.

2. Materials and methods

2.1. Experimental site

Cassava (*Manihot esculenta* cv. Rayong 9) was planted in the field plots during November 2015, which is the dry season period at Khon Kaen University (KKU), Khon Kaen Province, Northeastern region of Thailand (Lat. 16° 28′ 25″ N, Long. 102° 48′ 34″ E, Alt. 190 m). The upland soil was sandy loam (sand 57%, silt 31% and clay 12%, EC 0.36 dS m^{-1}, pH 6.0, CEC 5.90 cmol kg^{-1}, OM 1.12 g kg^{-1}, total N 1.93 g kg^{-1}, available P 2.80 mg kg^{-1} and available K 38.50 mg kg^{-1}) (Figure 1). The field capacity and permanent wilting point were 14.91%Vol and 4.55%Vol, respectively. Farm yard manure (4% N; 4% P and 4% K) at the rate of 6.25 ton ha^{-1} was applied before planting. The chemical fertilizer (15-7-18 N-P$_2$O$_5$-K$_2$O) was applied at 312.5 kg ha^{-1} twice after 1 and 2 months of plantation, as per the recommendation of good agricultural practices for cassava by Department of Agriculture, Thailand (Thai Agricultural Standard; TAS 5901-2010). Eighty-five days after planting (DAP), the plants were exposed to the water deficit stress (WS). Sprinkle irrigation was used for well-watered plots (WW) when the tensiometer was less than −30 kPa (at 20 cm depth from the soil surface) to maintain the soil water potential. Data for soil moisture, rainfall, air temperature, relative humidity, wind speed and solar radiation were collected from the weather station located within the field plots. During the growth period, the air temperature and relative humidity ranged between 9 and 44°C and 23 and 83%, respectively; the average solar radiation was 437 MJ m^{-2} and total amount of rainfall was 108 mm.

2.2. Field measurements

FLIR camera (model E50, spectral range 7.5–14.0 μm and resolution of 240 × 180 pixel) was placed 1.5 m above the top of the cassava canopy. The images were taken twice a month during February to April 2016 under clear sky between 11 am-2 pm in three plants per plot (afterwards taking the average) in both the WW and WS plots. Surface of the leaf in each plant was set for the wet reference (T_{wet}) by soaking both sides of the leaf in water and dry reference (T_{dry}) by covering both sides with petroleum jelly (Vaseline®) to prevent transpiration. The reference surface was used
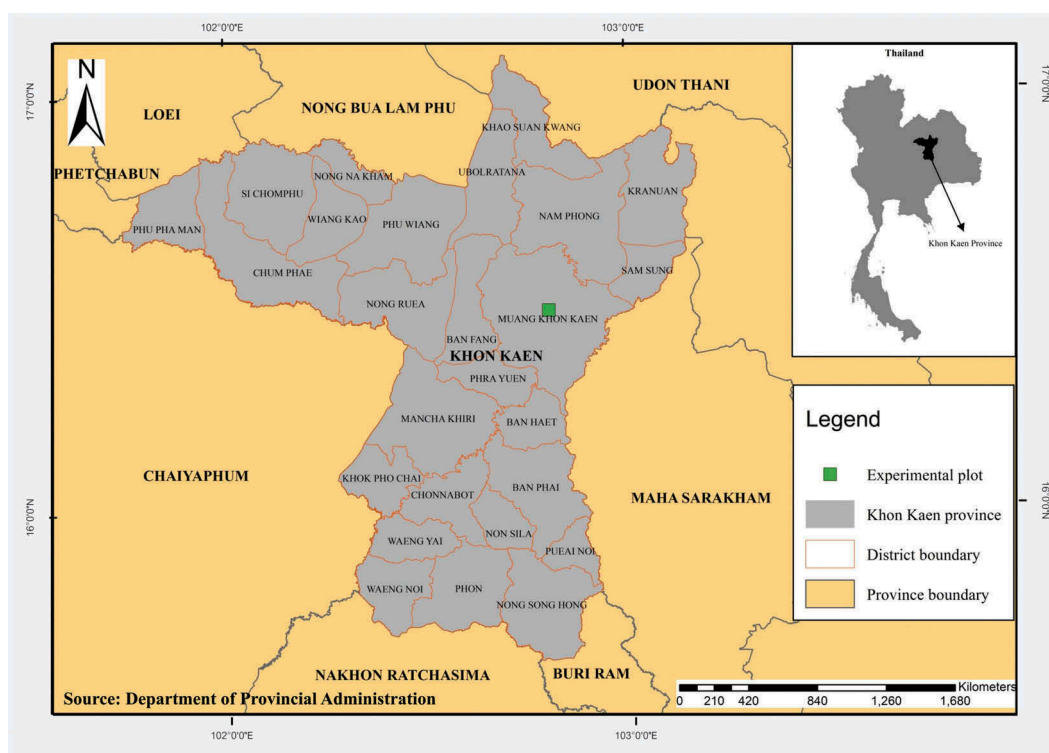
Figure 1. Location of the study area in Khon Kaen province, Thailand. The figure has been reproduced with permission from Department of Provincial Administration, Ministry of Interior, Thailand.

to eliminate errors and calibrate the sensor. The thermal images of WW and WS plants taken during the time of the experiment are presented in Figure 3. Net photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (T_r) and air vapor pressure deficit (VPD_{air}) were measured using portable photosynthesis system (model LI-6400XT, LI-COR Nebraska, USA). The water use efficiency (WUE) was calculated using P_n and T_r. The light intensity, chamber temperature, CO_2 concentration, and air flow rate of IRGA (infrared gas analyzer) chamber was maintained at 1000 µmol m^{-2} s^{-1} PPFD (photosynthetic flux density),

30°C, 380 µmol CO_2 mol^{-1} and 500 µmol s^{-1} air flow, respectively. The physiological parameters were measured on the same fully mature leaves used for thermal imagery within 10 min after the thermal image was captured. In addition, growth characters, that is, plant height, bush diameter and number of fallen leaves, were also measured (Table 1).

2.3. Experimental design and data analysis

The experiment was designed as a Completely Randomized Design (CRD) to draw comparison

Table 1. Shoot height, bush diameter and number of fallen leaves in cassava grown under well watered (WW) and water-stressed (WS) plots with three replications ($n = 3$).

			Day after planting			
Treatment	85	100	114	128	148	163
Height (cm)						
WW	103 ± 3.05	118 ± 4.26	128 ± 4.90	153 ± 4.90	170 ± 4.37	177 ± 4.26a
WS	102 ± 0.70	123 ± 2.52	123 ± 0.70	135 ± 1.21	148 ± 1.85	150 ± 2.43b
t-test	ns	ns	ns	ns	ns	*
Bush diameter (cm)						
WW	108 ± 1.36	105 ± 1.18	117 ± 0.68a	113 ± 1.80a	108 ± 1.80a	133 ± 1.36a
WS	107 ± 1.80	103 ± 0.68	95 ± 1.18b	97 ± 1.36b	88 ± 2.45b	83 ± 1.80b
t-test	ns	ns	**	*	*	**
Number of fallen leaves						
WW	9 ± 0.26	12 ± 0.23	14 ± 0.35	15 ± 0.70b	24 ± 0.93b	39 ± 1.19b
WS	6 ± 0.26	9 ± 0.26	11 ± 0.13	19 ± 0.96a	33 ± 0.23a	50 ± 0.35a
t-test	ns	ns	ns	**	*	*

Data presented as the mean of three replicates ($n = 3$) with standard error (± SE), and different letters in a column represent significant difference at $p \leq 0.05$. ns: not significance different, *: significant at $p \leq 0.05$, **: highly significant at $p \leq 0.01$ using *t*-test.

between well irrigation (WW) and water deficit stress (WD). Three replications ($n = 3$) were maintained for each treatment and three plant samples per treatment were used for the analysis. Mean value of each treatment was compared using t-test. Each plot site was 28 × 7 m^2 with 1 × 1 m^2 spacing between plants. FLIR images were captured and FLIR Tool 5.1 software was used for the image analysis. The distance between the camera and the plant canopy was set at 1.5 m and the selected emissivity value was 0.95. Canopy temperature (T_c) was determined as the average temperature, measured by a calculation of the canopy thermal imaging. Crop water stress index (CWSI) was calculated according to the equation given below (Jones & Schofield, 2008; Wiriya-Alongkorn et al., 2013).

$$CWSI = \frac{(T_c - T_{wet})}{(T_{dry} - T_{wet})}$$

where T_c is the mean canopy temperature, T_{dry} is the dry reference temperature obtained by coating leaves with petroleum jelly and T_{wet} is the wet reference temperature obtained by soaking leaves into the water. The CWSI value ranges between 0 and 1, in which 1 indicates maximum stress and 0 indicates the absence of stress. Data were analyzed in R statistical program and the means of CWSI, P_n, g_s, T_r, VPD$_{air}$ and WUE under different treatments were compared using t-test. The coefficient of determination (R^2), the significance of the correlation and relationship among P_n, g_s, T_r, VPD$_{air}$ and

CWSI were also calculated. The root mean square error (RMSE) of the studied physiological parameters was assessed as per the equation given below:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} \left(X_{obs,i} - X_{model,i}\right)^2}{n}}$$

where $X_{obs,i}$ refer to the observed samples at i, $X_{model,i}$ refer to the model samples at i and n refer to the number of samples

3. Results

3.1. Cassava growth and physiological adaptation in the water deficit conditions

Soil moisture in the well-watered plots (WW) was maintained, whereas it was declined in the water-stressed plots (WS) (Figure 2(f)). The average soil moisture at 20 cm depth under well-watered (WW) and water-stressed (WS) conditions was −9.26 and −23.98 kPa, respectively (Figure 2). At 140, 162 and 172 DAP (days after plantation), soil moisture and relative humidity in the field trials were increased due to the precipitation (Figure 2(e)), whereas the intensity of solar radiation was dropped (Figure 2(a)), because of the cloudy days. Shoot height of cassava during the initial period of stress (15–63 d water withholding period; 85–148 DAP) was maintained when compared with WW plants, while it was significantly decreased during late WS
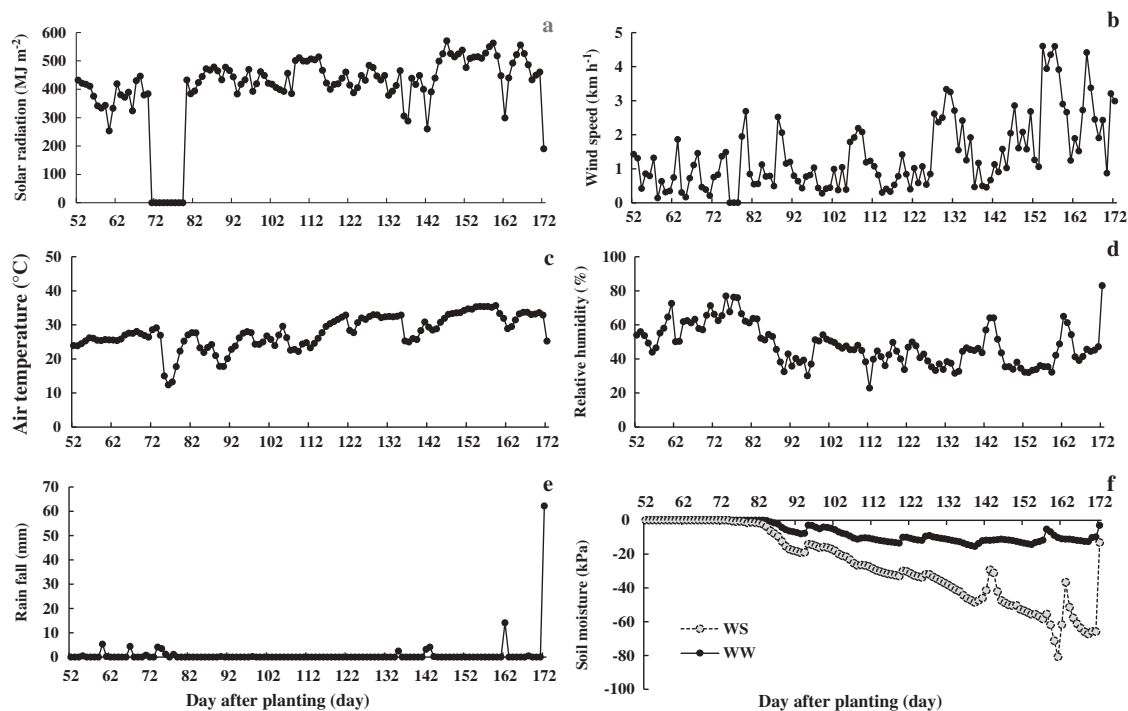

Figure 2. Daily average values of weather and soil moisture data during the days of experiment.
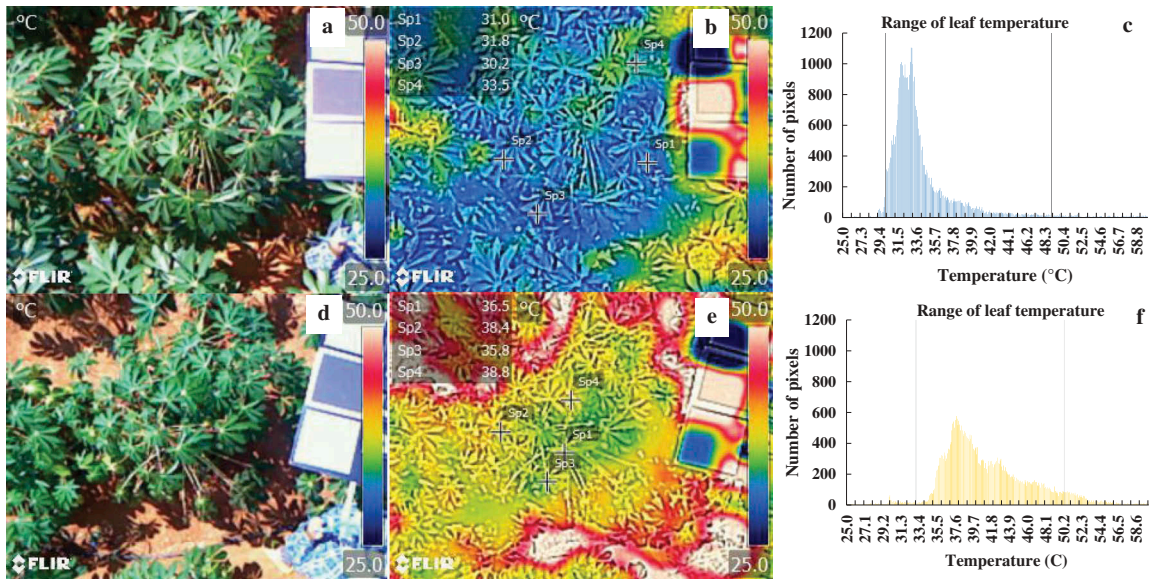
Figure 3. Morphological characters, thermal image and spectral emission of well-watered (a,b,c) and water-stressed (d,e,f) plants during the period of experiment. SP1 is 4th fully expanded leaf; SP2 is 5th fully expanded leaf; SP3 is a wet reference (T_{wet}) and SP4 is a dry reference (T_{dry}).

period (78 d water withholding period; 163 DAP) (Table 1). Bush diameter of cassava was declined when subjected to water deficit conditions for 63 d (148 DAP), and there was an increase in number of

fallen leaves after 43 d water withholding period (128 DAP) that continued till harvesting period (Table 1).

After water withholding (85 DAP), P_n, g_s and T_r in the mature leaf of cassava under WS were decreased, especially
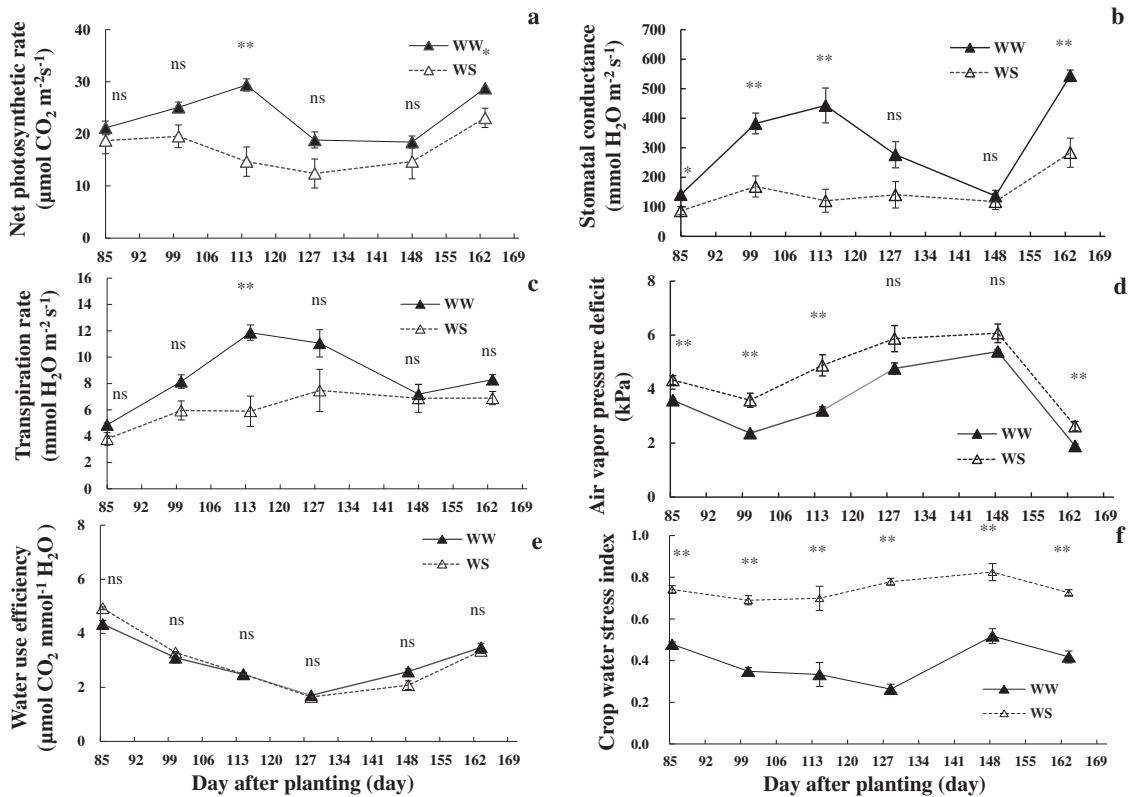

Figure 4. Net photosynthetic rate (a, P_n), stomatal conductance (b, g_s), transpiration rate (c, T_r), air vapor pressure deficit (d, VPD$_{air}$), water use efficiency (e, WUE) and crop water stress index (f, CWSI) of well-watered (WW) and water-deficit stressed (WS) plots of cassava. Data presented as the mean of three replicates with standard error (±SE), and different letters in each time point represent significant difference at $p \leq 0.05$. ^{ns}: not significance different, *: significant at $p \leq 0.05$, **: significant at $p \leq 0.01$ using t-test.

after the 29 d water withholding period (Figure 4(a)–(c)). Stomatal conductance was identified as the most sensitive parameter to WS, which was significantly dropped during the early water withholding period (15–29 d). In the late water deficit period, P_n, g_s and T_r were increased, depending on the precipitation, especially when the transpiration rate of WS plants was equal to WW plants (Figure 4(c)). In contrast, VPD_{air} was higher in case of water deficit conditions than well-watered situations, and subsequently after rainfall it turned to be insignificantly different due to a decrease in evaporation rate because of high humidity and cloudy conditions (low solar radiation) (Figure 4(d)). WUE did not vary significantly between WW and WS (Figure 4(e)).

3.2. CWSI in cassava under different water regimes

CWSI in cassava plants under water limited conditions was higher than control throughout the water withholding period (Figure 4(f)). The CWSI values of the WW and WS plants were 0.39 and 0.74, respectively. The critical CWSI value was 0.60, which was determined by a ratio of the WW and WS plants. In extreme water deficit conditions, the P_n, g_s and T_r in cassava were decreased in response to the WS, while the VPD_{air} was gradually increased.

3.3. Calibration and validation between plant physiology and CWSI

Negative correlation was demonstrated between the CWSI and P_n ($R^2 = 0.34$; $p \leq 0.05$), g_s ($R^2 = 0.36$; $p \leq 0.05$) and T_r ($R^2 = 0.43$; $p \leq 0.05$) (Figure 5). The P_n, g_s and T_r decreased gradually with increased CWSI. Conversely, the value of CWSI was positively correlated with VPD_{air} ($R^2 = 0.23$; $p \leq 0.05$) under water deficit conditions. The relationship between CWSI and T_r was found to be the strongest in a linear regression ($-8.5346\times + 12.207$, $R^2 = 0.43$), followed by g_s ($y = -468.28x + 501.76$, $R^2 = 0.36$), P_n ($y = -18.51x + 30.631$, $R^2 = 0.34$) and VPD_{air} ($y = 3.4257x + 2.1015$, $R^2 = 0.23$) (Figure 5). The RMSE physiological data was fit. The CWSI value was validated for the VPD_{air} with the RMSE = 0.96 and $R^2 = 0.80$. The P_n and g_s were also found a good correlation with RMSE 3.56 and 52.26, $R^2 = 0.77$ and $R^2 = 0.76$, respectively (Table 2).

Table 2. The calibrated model and validated physiological parameter of the cassava under different water regimes.

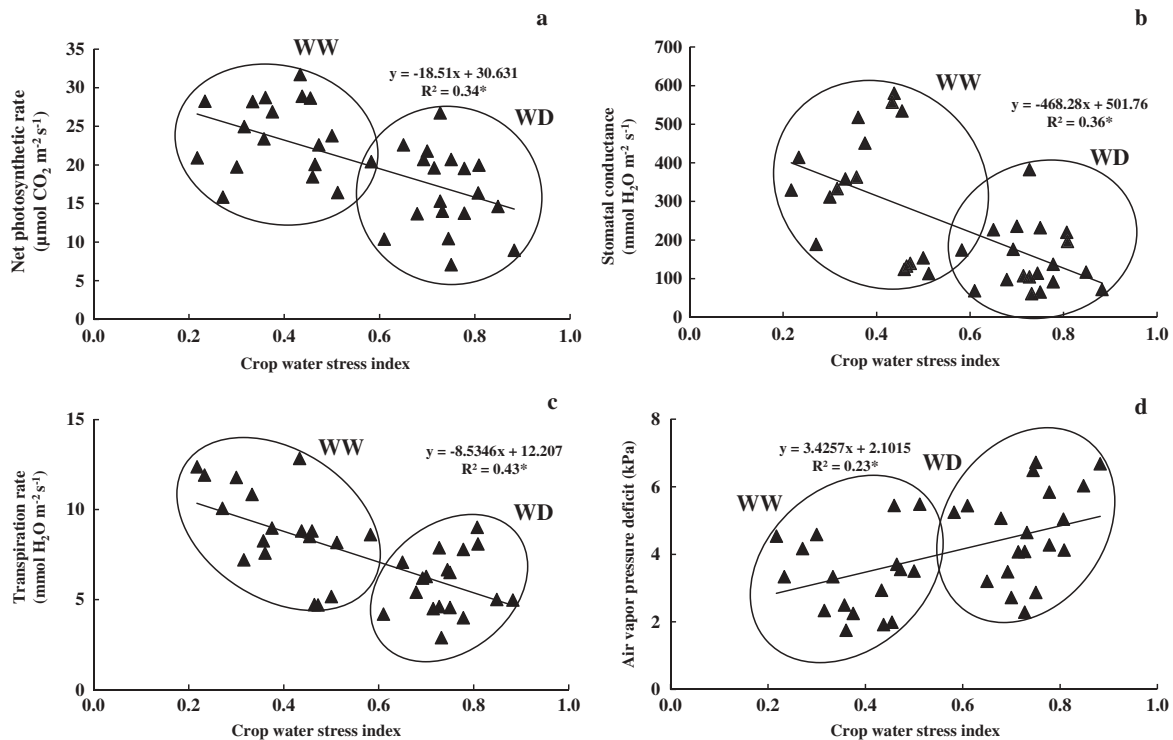| Output variable	Calibrated Model expression	Validated RMSE	Validated R-squared
P_n	$y = 30.63 - 18.51x$	3.56	0.77
g_s	$y = 501.76 - 468.28x$	52.26	0.76
T_r	$y = 12.21 - 8.54x$	2.25	0.52
VPD_{air}	$y = 2.10 + 3.43x$	0.96	0.80

Figure 5. Correlation between CWSI and net photosynthetic rate (a, P_n), stomatal conductance (b, g_s), transpiration rate (c, T_r) and air vapor pressure deficit (d, VPD_{air}) in cassava stressed and nonstressed environments.

4. Discussion

Precipitation, rate of evaporation and relative humidity were identified as the key factors that controlled the soil moisture in the experimental plots. An earlier study validated that the variations in rainfall patterns at different sites ('Rihacha' semiarid environment and 'Santos' seasonally dry environment in Colombia) can be used as drought treatment for cassava field trials (de Tafur, El-Sharkawy, & Calle, 1997). In cassava, number of leaves in drought stressed plants cv. 'Nyalanda' (drought susceptible) was significantly declined (by 37.8%) to that of WW plants, whereas plant height was unchanged when exposed to water deficit stress for 10 d (Turyagyenda et al., 2013). Leaf number and above ground biomass of cassava cv. 'MCol 1468' under prolonged drought conditions (25% field capacity or 28 d water withholding) were decreased by 49.0% and 56.5%, respectively, when compared with the control (Vandegeer et al., 2013). In addition, stem height in cassava cv. 'CM 1585-13' under low water availability (80 cm^3 irrigation volume) was retarded by 31.2% and the leaf fall was induced by 2.17 folds over high water availability (1200 cm^3 irrigation volume) (Calatayud et al., 2000). Leaf senescence or leaf fall in drought stressed cassava plants was considered a good visual indicator for the drought responses, and can be related to the degree of increasing water-deficit stress (Liao et al., 2017). In addition, the storage root of cassava was identified as the most affected part when plants are subjected to drought conditions (de Tafur et al., 1997; El-Sharkawy, 2007).

In the present investigation, CO_2 assimilation and stomatal conductance in cassava cv. 'Rayong 9' under WS were significantly declined and acted as the most sensitive indicators during the early water withholding period of 29 d. Similarly, P_n and g_s in cassava cv. CM 1585-13 (4 months old) under low water availability were significantly declined by threefolds over well irrigated plants (Calatayud et al., 2000). P_n, g_s and T_r in 8 cultivars of cassava (60 DAP) under prolonged WS for 120 d were decreased during the early period of stress (within 30 d after water withholding) (El-Sharkawy, 2007). Moreover, P_n, g_s and T_r were significantly reduced in cassava cv. CM 507-37 grown in the pot culture with reducing fraction of transpirable soil water (FTSW) for 7 d when compared with the regularly irrigated plants (Cruz et al., 2016). The g_s in cassava cvs. 'Nyalanda' (drought susceptible) and 'MH96/0686' (drought tolerant) exposed to WS for 10 d was significantly declined by 27.9% and 51.7% to that of WW plants, respectively (Turyagyenda et al., 2013). In addition, the evaporation rate and relative humidity in the field trial environments were directly related to rainfall and solar radiation,

leading to reduce the VPD_{air}, especially during the prolonged drought (de Tafur et al., 1997).

The correlation between T_r and CWSI could be used to estimate the cassava water stress level ($R^2 = 0.43$). The CWSI was inversely related to T_r, rather than to g_s (Jones, 2014). The P_n, g_s and T_r of cassava were more sensitive to the water stress than the VPD_{air}. Cassava was more sensitive to WS when atmospheric humidity and soil water status were changed (El-Sharkawy & Cock, 1984). In the dry air, stomatal pores in cassava leaves were closed to prevent water loss (Okogbenin et al., 2013). The air temperature and relative humidity, therefore, highly affected the P_n, g_s, T_r and VPD_{air} in cassava and the WW cassava plot was rapidly acclimatized to the high air temperature and low relative humidity. The g_s, P_n and T_r in cassava under WS were decreased, in relation to the increased VPD_{air}. Subsequently, these changes promoted the CWSI.

The appropriate time to determine the CWSI is between 11 am to 2 pm. In early morning, the relative humidity around the plant canopy was still enriched, leading to similar value of CWSI in plants under WW and WD conditions, whereas it was declined, in relation to the high evaporation rate in mid-day and late afternoon period (Zia et al., 2011). In general, stomatal closure is evidently observed to prevent the excessive water loss via transpiration during the afternoon time witnessing high air temperature, air ventilation, low relative humidity and soil water deficit (Grant, Chaves, & Jones, 2006; Wiriya-Alongkorn et al., 2013). Therefore the measurements were carried out at near midday under clear sky and since hot dry air might affect the temperature measurements (Jones, 2014), the error in the temperature measurement was removed from the number of samples by obtaining a good correlation. An accurate determination of correlation between CWSI and physiological parameters requires rapid monitoring. In the present study, the critical CWSI in cassava under prolonged drought was estimated to be 0.60, which could be used for timely planning of the irrigation. Similarly, a study validated that the CWSI and water deficit index (WDI) in corn was based on vapor pressure deficit using remote sensing (Tanriverdi et al., 2017). This research revealed the application of thermal imaging to assess cassava physiological responses towards the water deficit stress during storage root initiation to the time of final yield harvest, identifying as drought critical stage in cassava cultivation (leading to yield drop by 32–60%) (Hillocks et al., 2002). Also, thermal sensing is a simple, rapid, reproducible, non-destructive and acceptable technique, linked to the physiological parameters, e.g. stomatal conductance, plant

water status, root performance and storage root yield attributes (Pask et al., 2011).

5. Conclusions

Decreased morphological and physiological parameters such as shoot height, bush diameter, net photosynthetic rate, stomatal conductance and transpiration rate and increased air vapor pressor deficit and CWSI showed their sensitivity to the water deficit stress. In addition, the close relationships between CWSI and transpiration rate, stomatal conductance, and net photosynthetic rate were demonstrated that explains the strategies adapted by cassava during water deficit. Further research should focus on exploring other remote sensing imagery options, particularly satellite data, for monitoring the large fields.

Disclosure statement

No potential conflict of interest was reported by the authors.

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