Non-Destructive Leaf Area Estimation Model for Overall Growth Performances in Relation to Yield Attributes of Cassava (Manihot esculenta Cranz) under Water Deficit Conditions

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Abstract
Cassava is a tropical storage root crop, a source of carbohydrate and alternative energy. It has been classified as “drought tolerant plant” for the whole life cycle, except during the root initiation stage (120-150 DAP). Leaf area index (LAI) is one of the most parameters representing the overall growth and yield prediction in cassava. The aim of this investigation was to validate the physiological and growth performance of cassava in response to water deficit stress in the field trial as well as to investigate the leaf area index as an important factor to cassava growth and storage root bulking. Leaf relative water content in cassava declined significantly upon a long period of water withholding, and regulated non-photochemical quenching (NPQ), leading to chlorophyll degradation, reduced number of leaves and limited leaf area index (LAI) and loss of storage root yield when compared with well-irrigated plants. Non-destructive leaf area estimation model under water deficit stress condition using spectral reflectance to determine the LAI and VIs was validated. The Ratio Vegetation Index (RVI) was suitable model with high coefficient of determination ($R^2 = 0.89$). However, the RVI as LAI at 150 DAP (120 d water withholding) could be considered as the critical point to indicate cassava growth and yield performance. Based on the results, cassava growth, biomass and yield in the different environments may further be investigated, taking into consideration the genotypic variation and using remote sensing technology for rapid monitoring and accurate and cost-effective data assessment.

Keywords: cassava; growth performances; leaf area index; non-destructive model; vegetation indices

Introduction
Loss in the yield of crops such as cereals, legumes, tubers and root crops due to the drought incidents borne out of global climate change are evidently demonstrated in several regions of the world (Daryanto et al., 2017). In tuber crops (potato and cassava), the storage root initiation is more sensitive to drought stress than vegetative developmental stages (Daryanto et al., 2016). Cassava (Manihot esculenta Cranz), one of the top five crops used for global starch production, played a key role in bio-based fuel as bioethanol for 150 L tonne$^{-1}$ or 6,000 L ha$^{-1}$ year$^{-1}$ (Jansson et al., 2009; Ziska et al., 2009; Numamanya et al., 2012), especially in Thailand (3.4 million litres per day; Nguyen et al., 2007). Previously, cassava has been identified as a drought tolerant plant that can withstand the dry season for 4-6 months, depending upon the evaporation rate (Oguntunde, 2005), available soil water (irrigation schedule) (Pardales and Esquibel, 1996), and genotypic variation (El-Sharkawy, 2007; Okogbenin et al., 2013). However, it was reported by many physiological studies that net photosynthetic rate, stomatal conductance, transpiration rate and water use efficiency in cassava grown under drought conditions significantly declined when compared with well-irrigated plants (Itani et al., 1999; El-Sharkawy, 2007). In addition, overall growth performances of both shoot and root traits are affected, depending on the level of drought stress (El-Sharkawy, 2007; Duque and Setter, 2013).

Leaf area, leaf retention and leaf area expansion rate in cassava is related to its productivity, especially during the water shortage (Alves and Setter, 2000; Alves and Setter, 2004; Lenis et al., 2006). Leaf area index (LAI) is an indicator of leaf area per unit ground area (Jonckheere et al., 2004) and is regulated by the genotype, plant age,
environment, management practices and cropping system (Ekanayake et al., 1998). In cassava, leaf area is a key indicator of crop growth rate and the storage bulking rate (Cock et al., 1979a). Leaf area of cassava depends on number of branches, number of leaves, leaf expansion and leaf retention. In general, the leaves are produced within 90 to 120 days after plantation (DAP) and the maximum total leaf area is achieved within 120 to 150 DAP. The rate of leaf formation is decreased depending on plant developmental stage from initiation to maturity. An optimal leaf area index (LAI) for storage root bulking is 3.0 to 3.5 m$^2$ m$^{-2}$ ground area (Hillock et al., 2001). In Thailand, the optimal LAI for starch formation is 4.0 m$^2$ m$^{-2}$ as well as the net photosynthetic rate decreases depending upon the level of shade (Boonseng, 2008). In a recent study, simulating impact model of cassava grown under drought stress (short-, long-water stress and recovery) has been reported using the LINTUL in relation to LAI (Ezui et al., 2018).

Non-destructive Leaf Area Estimation model has been investigated and validated in several crop species, such as jatropha (Pompre et al., 2012), kiwi (de Gyves et al., 2007), hazelnut (Cristofori et al., 2007), Persian walnut (Kera matlou et al., 2015), apple (Sala et al., 2015), teak (Tondjo et al., 2015), soybean (Bakhshandeh et al., 2011) and fava bean (Peksen, 2007). In two morphotypes of cassava (Philippine and Nagra), linear regression models of leaf number prediction have been validated (Fakir et al., 2011). Several methods of LAI measurements have been developed, directly from the leaf area measurements or indirectly through a non-destructive measurement. Direct methods are accurate but labor intensive and therefore, have a limited use. When the number of plants is limited, the estimation of LAI using non-destructive method is better, simple, quick, accurate, reliable and inexpensive procedure (Wiersma and Bailey, 1975). In addition, the remote sensing has been used to estimate LAI and it changes to the broadband spectrum (Boonyanuphap, 2014). Spectral indices or vegetation indices (VIs) are the new variables generated by mathematical combinations of two or more original spectral bands of the biophysical parameters such as canopy LAI, water content, pigment, etc. (Jone and Vaughan, 2010; Reynolds et al., 2011). The relationship between Normalized Difference Vegetation Index (NDVI) and LAI in case of paddy rice, pearl millet sugar cane, pasture, corn, eucalypt and riparian forest were found to be the best potential models (Xavier and Vettorazzia, 2004; Vijendra and Chandra, 2012; Xiao et al., 2012). The Normalized Difference Water Index (NDWI), NDVI and the Gitelson-Merzylik Index (GMI) of almonds also showed a high correlation with LAI (Jose et al., 2012). Moreover, the studies based on the variations in leaf area values under salinity and water stress conditions in green pepper, tomato and cucumber have also been undertaken (Cemek et al., 2011; Hossain et al., 2017). Therefore, the best estimated model in cassava crop has still been limited in the literatures. The present study has been conducted to investigate the crop responses towards prolonged drought conditions. We used VIs for estimated LAI of cassava to indicate overall growth performances and yield attributes in the field trial under well-irrigation and water deficit condition.

**Materials and Methods**

**Experimental site and design**

The experiment was carried out at the Thai Tapioca Development Institute area, Nakhon Ratchasima province, Thailand (15°09'09.8"N, 101°29'42.8"E) between February - December 2014. Cassava [Manihot esculenta cv. ‘Huay Bong 80’ (KU50 × Rayong 5) yield = 30.6 - 34.4 ton ha$^{-1}$, and starch content = 27.3% w/w; Vichukit et al., 2011] was planted in the field. The experimental design was a completely randomized design (CRD) under well-watered (WW) and water-stressed (WS) conditions with three replications in a 16 × 16 m plot. The plant and row spacing was maintained 0.8 and 1.2 m, respectively. Soil texture of the area is sandy clay loam in the top 0.50 m (sand 59%, silt 16%, clay 25%, pH 6.7, EC, 0.39 dS m$^{-1}$, OM 1.15%, available P 31.85 mg kg$^{-1}$ and exchangeable K 161.99 mg kg$^{-1}$). The moisture at field capacity and permanent wilting point were 29.12 and 11.49 %Vol, respectively. The chemical fertilizer (15-7-18 N-P-O5-K2O) was used at the rate of 312.5 kg ha$^{-1}$ at 1 month after plantation (MAP). The online soil moisture sensors (ML3 ThetaProbe Soil Moisture Sensor, AT Delta-T Devices, London UK) were installed at 15 cm depth from soil surface in WW and WS plots for estimating the soil moisture enclosing the cassava root zone. WS plot was applied with the sprinkle irrigation at weekly intervals up to 1 MAP and thereafter, no irrigation was provided until harvesting process. WW plot was irrigated with the sprinkle (2 m$^2$ h$^{-1}$ for 2 h) to maintain soil moisture at 80% of available water (AW) or 25 %Vol during the whole experiment (10 d interval using 4 m$^3$ per 6.28 m$^2$). The weather station was installed near the experimental plot to collect the solar radiation, wind speed, air temperature, air relative humidity and rainfall that ranged from 84 to 348 MJ m$^{-2}$, 1 to 19 km h$^{-1}$, 25 to 31 °C and 63 to 93% RH, respectively. The total rainfall during the field trial was 690 mm (Fig. 1).

**Field measurements**

LAI were measured every month from 60 DAP until 240 DAP using LI-COR LAI-2000 Plant Canopy Analyzer (Fig. 2). The LAI-2000 was used as a fisheye light sensor that measured by making a reference reading above the canopy (sensor aimed up at the sky), and one or more readings beneath the canopy (sensor again looking up). The below canopy readings were divided by the above canopy readings to obtain an estimate of the gap fraction at the five angles. The sensors were always shaded from direct sun when in use (Welles, 1990). LAI was computed using the ellipsoidal inversion model and integrated into the LAI-2000 software program, and easily applied to the PAR data collected in the field (Peper and McPherson, 1998). In this research, LAI measurements were conducted within the middle five rows of the plot using one-above and four-below canopy readings. The LAs were calculated to represent each plot and were used for the estimation and validation of the model.

Measurements of spectral reflectance were started from 60 DAP and continued until 240 DAP. The measurements were carried out over two data set using Field Spectroradiometer, FieldSpec 3 (ASD), within a spectral
range of 350 to 2,500 nm. Measurement has also defined field of view (FOV) at 25°FOV at 1.5 m that cover above the canopy and ground about 0.67 m. Within each plot, the spectral reflectance was measured 6 plants during 11.00h to 14.00h, on a clear sunny day (Reynolds et al., 2011; Pask et al., 2012). The average reflectance values were calculated as the estimated VIs for estimation and validation of the model.

Relative water content (RWC) was measured in the fully expanded leaves. Leaf discs of about 5-10 cm² were cut from each sample and weighed to obtain the fresh weight (FW). After that the samples were immediately hydrated to full turgidity for 4h under room conditions. After 4 h, the samples were taken out of the water, well dried of any surface moisture quickly and lightly with a filter paper and immediately weighed to obtain fully turgid weight (TW). Samples were then oven-dried at 80 °C for 24h and weighed after cooling down to determine dry weight (DW). The RWC was calculated as:

\[
\text{RWC (\%)} = \left(\frac{\text{FW} - \text{DW}}{\text{TW} - \text{DW}}\right) \times 100
\]

where the FW is fresh weight, TW is turgid weight and DW is dry weight of sample.

Leaf greenness in the second expanded leaf of both WW and WD treatments in each time point was measured using SPAD-502Plus (Konica Minolta, Japan) following the method of Uddling et al. (2007).

Chlorophyll fluorescence emission was measured from the adaxial surface of second expanded leaf using a fluorescence monitoring system (model FMS 2; Hansatech Instruments Ltd., Norfolk, UK) in the pulse amplitude modulation mode (Loggini et al., 1999). A leaf, kept in dark for 30 min, was initially exposed to the modulated measuring beam of far-red light (LED source) with typical peak at a wavelength of 735 nm. Original (F₀) and maximum (Fₘ) fluorescence yields were measured under weakly modulated red light (<85 µmol m⁻² s⁻¹) with 1.6 s pulses of saturating light (>1,500 µmol m⁻² s⁻¹ PPFD) and calculated using FMS software for Windows®. The variable fluorescence yield (Fᵥ) was calculated using the equation: Fᵥ=Fₘ-F₀. The ratio of variable to maximum fluorescence (Fᵥ/Fₘ) was calculated as the maximum quantum yield of PSII photochemistry. The photon yield of PSII (Φₘ) in the light was calculated as: Φₘ = (Fₘ-F₀)/Fₘ after 45 s of illumination, when steady state was achieved as well as non-photochemical quenching (NPQ) was archived (Maxwell and Johnson, 2000).

Growth including plant height and number of leaves per plant were measured 30 DAP and continued until 240 DAP in 6 samples per plot. Biomass and yield were manually determined at the harvesting time (300 DAP).

Data analysis

The spectral reflectance calculated for the VIs was in the red (640-670 nm), the green (530-590 nm), the blue (450-510 nm) and the near infrared (850-880 nm). Based on previous studies, six VIs having a good correlation to LAI using Pearson’s correlation coefficient are: (1) Normalized Difference Vegetation Index (NDVI), (2) Ratio Vegetation Index (RVI), (3) Soil-Adjusted Vegetation Index (SAVI), (4) Enhanced Vegetation Index (EVI), (5) Visible Atmospherically Resistant Index (VARI), and (6) Modified Second Triangular Vegetation Index (MTVI2) (Fig. Supplementary 1). Evaluated equations in each index were regarded by model validation as estimated values, following previous studies (Table 1).

The correlation analysis of LAI and VIs was analysed statistically using the coefficient of determination (R²) using R statistical program (Fig. Supplementary 2) and the simple linear regression by Pearson’s correlation coefficient (Fig.

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Fig. 1. Rainfall (a), wind speed (b), solar radiation (c), air temperature (d), relative humidity (e) and soil moisture content (f) in the field trial of cassava.
Supplementary 3). Mean values in each parameter were compared using t-test. The root mean square error (RMSE) for the measured LAI was assessed using the equation below.

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

where $X_{\text{obs}}$ refer to the observation samples at $i$, $X_{\text{model}}$ refer to the model samples at $i$ and $n$ refer to the number of samples. Calibrated calculated model using exponential equation was validated (Fig. Supplementary 2).

Results

Growth performances, physiological changes and yield attributes in response to water deficit

Number of leaves in cassava was significantly dropped in plants grown under water deficit conditions (Fig. 3a). At 150 DAP (120 d water withholding), number of leaves in water deficit stressed plants was 15.34% lower than that in WW plants (Table 2). It was evidently demonstrated that the number of fallen leaves was promoted by adaptive period of water deficit, leading to decrease LAI parameter. LAI in both WW and WS conditions increased in relation to the growing period. In addition, the reduction percentage of LAI in WS cassava at 150 DAP was 34.32% lesser than WW plants (Table 2). Shoot height was represented as overall growth performances of cassava, which was a sensitive parameter to water deficit stress. Shoot height in cassava was significantly increased with the developmental stages in both WW and WS treatments (Fig. 3c). In water deficit stressed plants at 150, 180 and 210 DAP, shoot height was significantly declined by 31.63%, 13.99% and 15.43%, respectively (Table 2). RWC in the leaf tissues was maintained in WW plants, whereas it was significantly declined by 21.44%, 29.23% and 31.33% when exposed to water withholding for 90 (120 DAP), 120 (150 DAP) and 150 d (180 DAP), respectively (Table 2 and Fig. 3d).

Total chlorophyll content or greenness of leaves was retained in the early period (60-120 DAP) of water withholding and subsequently degraded by 8.17%, 4.44%, 6.31% and 9.90% when exposed to water withholding for 150, 180, 210 and 240 d, respectively (Fig. 4a). The degradation rate of chlorophyll pigment depended on the long period of water deficit condition, causing chlorosis symptom prior to leaf senescence, especially older leaf position. In contrast, the maximum quantum yield of PSII ($F_{v}/F_{m}$) and photon yield of PSII ($\Phi_{PSII}$) in all treatments and at all the time periods were unchanged (Fig. 4b and 4c). In general, the $F_{v}/F_{m}$ and $\Phi_{PSII}$ were sensitive to water deficit.
deficit, depending on plant species, genotypes, a degree of water deficit stress and their interaction. In present study, those parameters were retained relating to the highly efficient adaptation of cassava to water limitation. Interestingly, non-photochemical quenching (NPQ) in the water deficit stressed plants was significantly increased by 1.32 (150 DAP) and 1.90 folds (180 DAP) over well-irrigated plants (Fig. 4d), indicating the activity of anti-photooxidative generation.

At the harvesting stage (300 DAP), whole plant was manually collected and weighed separately as fresh storage root (kg plant$^{-1}$) and the above ground biomass of stem and leaves (Fig. 5). Total above ground biomass, number of storage roots and storage root fresh weight in cassava grown under water shortage were significantly declined by 57.57%, 18.18% and 30.41% to that of well-irrigated plants (Table 3).

**Table 2.** Number of leaves, leaf area index, plant height, and relative water content of cassava under WS and WW plots at 150 DAP. Value in parenthesis represents the % reduction between WS and WW conditions

<table>
<thead>
<tr>
<th>Water regime</th>
<th>Number of leaves (leaves plant$^{-1}$)</th>
<th>Leaf area index (m$^2$ m$^{-2}$)</th>
<th>Plant height (cm)</th>
<th>Relative water content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>80±1.84a</td>
<td>4.72±0.67a</td>
<td>163±6.17a</td>
<td>87.9±1.28a</td>
</tr>
<tr>
<td>WS</td>
<td>74±2.78b (7.50%)</td>
<td>3.10±0.23b</td>
<td>138±2.43b</td>
<td>60.1±1.02b</td>
</tr>
<tr>
<td>$t$-test</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>**</td>
</tr>
</tbody>
</table>

Data presented as mean ± SE and different letters in a column represent significant difference at $p \leq 0.05$. ns: not significant, *: significant at $p \leq 0.05$, **: highly significant at $p \leq 0.01$ using $t$-test.

**Table 3.** Total above ground biomass, number of storage roots and storage root fresh weight of cassava under WS and WW plots at the harvesting stage (300 DAP). Value in parenthesis represents the % reduction between WS and WW conditions

<table>
<thead>
<tr>
<th>Water regime</th>
<th>Total above ground biomass (kg plant$^{-1}$)</th>
<th>Number of storage root (storage roots plant$^{-1}$)</th>
<th>Storage root fresh weight (kg plant$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WW</td>
<td>3.70±1.40a</td>
<td>11±0.58a</td>
<td>4.44±0.13a</td>
</tr>
<tr>
<td>WS</td>
<td>1.57±0.70b (57.57%)</td>
<td>9±0.33b</td>
<td>3.09±0.46b</td>
</tr>
<tr>
<td>$t$-test</td>
<td></td>
<td>*</td>
<td>**</td>
</tr>
</tbody>
</table>

Data presented as mean ± SE and different letters in a column represent significant difference at $p \leq 0.05$. ns: not significant, *: significant at $p \leq 0.05$, **: highly significant at $p \leq 0.01$ using $t$-test.

Fig. 3. Number of leaves per plant (a), leaf area index (b), plant height (c), and relative water content in the leaf tissues (d) of cassava grown in well-watered (WW) and water-stressed (WS) plots. Data presented as the mean of three replicates with standard error (±SE), and different letters in a column represent significant difference at $p \leq 0.05$. ns: not significant, *: significant at $p \leq 0.05$, **: highly significant at $p \leq 0.01$ using $t$-test.
Fig. 4. Leaf greenness (SPAD; a), maximum quantum yield of PSII (Fv/Fm; b), photo yield of PSII (ΦPSII; c) and non-photochemical quenching (NPQ; d) of cassava grown under well-watered (WW) and water-stressed (WS) plots. Data presented as the mean of three replicates with standard error (±SE), and different letters in a column represent significant difference at p ≤ 0.05. ns: not significant, *: significant at p ≤ 0.05, using t-test.

Fig. 5. Manually harvesting method of storage root (a), harvested storage root of cassava (b), the above ground biomass and storage root morphological characters of cassava grown under well-watered (WW) (c) and water-stressed (WS) plots (d) for 10 months (300 DAP).
Vegetation indices to estimate leaf area index (LAI) in the water deficit condition

In the calibration model, LAI and VIs, non-destructive plant material methods under different water regimes were measured.

A significant difference of LAI at 120 and 150 DAP (after water withholding 90 and 120 d), was demonstrated and the range of LAI of WS plot was found to be less than 4 m² m⁻² ground area whereas it was more than 4 m² m⁻² in WW plot (Fig. 3b). Maximum LAI under WS and WW plot was measured to be 4.65 and 5.20 m² m⁻², at 210 DAP, respectively. The LAI gradually increased during the early growth stage up to 210 DAP during the canopy development stage, and thereafter, declined until the storage root was fully developed at 240 DAP. In present study, the Visible Atmospherically Resistant Index (VARI) in water deficit stressed plants was declined at the early stage (60 d water withholding) [27.7% reduction of control] and 90 d (22.81% reduction of control) water withholding period (Table 4). In addition, the Ratio Vegetation Index (RVI) was found to be a good indicator, which showed a significant difference at 90 DAP (19.33% reduction of control) and 210 DAP (21.26% reduction of control), 120 DAP (24.49% reduction of control) water withholding period, [27.27% reduction of control] water withholding period, (27.7% reduction of control) water withholding period, and the range of LAI of WS plot was found to be less than 4 m² m⁻² ground area whereas it was more than 4 m² m⁻² in WW plot (Fig. 3b). Maximum LAI under WS and WW plot was measured to be 4.65 and 5.20 m² m⁻², at 210 DAP, respectively. The LAI gradually increased during the early growth stage up to 210 DAP during the canopy development stage, and thereafter, declined until the storage root was fully developed at 240 DAP. In present study, the Visible Atmospherically Resistant Index (VARI) in water deficit stressed plants was declined at the early stage [60 d water withholding] [27.7% reduction of control] and 90 d (22.81% reduction of control) water withholding period (Table 4). In addition, the Ratio Vegetation Index (RVI) was found to be a good indicator, which showed a significant difference at 90 DAP (19.33% reduction of control), 120 DAP (24.49% reduction of control), 150 DAP (14.43% reduction of control) and 210 DAP (21.26% reduction of control) under water deficit conditions, depending on a reduction of plant canopy adaptation. In contrast, Normalized Difference Vegetation Index (NDVI), Enhanced Vegetation Index (EVI), Soil-adjusted Vegetation Index (SAVI) and Modified Second Triangular Vegetation Index (MTVI) models were insignificantly different between WW and WS using t-test (Table 4). The cassava LAI (Fig. 3b) and the six VIs (Table 1) showed highly positive exponential growth relationship and model equation (Table 5). The RVI and LAI showed the highest relationship under WS and WW plots with R² = 0.88 and R² = 0.82, respectively. The relationship between LAI and VIs of the cassava growth from 60 to 240 DAP was validated. The results showed that the RVI was obtained as the best index for estimating LAI under WS and WW plots with RMSE = 0.53 and 0.64, and R² = 0.89 and 0.75, respectively (Table 5).

Relationship between vegetation indices and yield attributes

At 90 and 120 d water withholding, the growth and physiological characters as well as LAI were significantly declined resulting in the reduction of final storage root yield. Based on this hypothesis, a positive relation between number of leaves and number of storage roots was demonstrated with R² = 0.99 at 120 DAP (Fig. 6a) and R² = 0.90 at 150 DAP (Fig. 6b). Moreover, a positive relation between LAI and storage root fresh weight was demonstrated with R² = 0.99 at 120 DAP (Fig. 6c) and R² = 0.92 at 150 DAP (Fig. 6d).

Table 4. Calibrated data set of cassava LAI and VIs under different water regimes. Value in parenthesis represents the % reduction between WS and WW conditions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day after planting</th>
<th>NDVI</th>
<th>SVI</th>
<th>RVI</th>
<th>EVI</th>
<th>SAVI</th>
<th>MTVI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60</td>
<td>90</td>
<td>120</td>
<td>150</td>
<td>180</td>
<td>210</td>
<td>240</td>
</tr>
<tr>
<td>WW</td>
<td>0.69±0.01</td>
<td>0.91±0.00</td>
<td>0.92±0.00</td>
<td>0.92±0.00</td>
<td>0.91±0.01</td>
<td>0.91±0.01</td>
<td>0.90±0.01</td>
</tr>
<tr>
<td>WS</td>
<td>0.67±0.05</td>
<td>0.89±0.01</td>
<td>0.90±0.01</td>
<td>0.91±0.01</td>
<td>0.92±0.00</td>
<td>0.93±0.00</td>
<td>0.92±0.00</td>
</tr>
<tr>
<td>t-test</td>
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<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>RVI</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td>5.39±0.15</td>
<td>21.00±1.12</td>
<td>25.68±0.69</td>
<td>25.01±1.22</td>
<td>24.55±2.30</td>
<td>27.19±0.80</td>
<td>20.24±2.19</td>
</tr>
<tr>
<td>WS</td>
<td>5.39±0.94</td>
<td>16.94±0.93</td>
<td>19.39±2.4b</td>
<td>21.40±1.4b</td>
<td>25.02±1.47</td>
<td>21.41±1.4b</td>
<td>23.20±0.77</td>
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<tr>
<td>t-test</td>
<td>ns</td>
<td>*</td>
<td>*</td>
<td>ns</td>
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<tr>
<td>EVI</td>
<td></td>
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</tr>
<tr>
<td>WW</td>
<td>0.52±0.01</td>
<td>0.83±0.01</td>
<td>0.96±0.02</td>
<td>1.00±0.01</td>
<td>0.98±0.02</td>
<td>0.91±0.01</td>
<td>0.87±0.03</td>
</tr>
<tr>
<td>WS</td>
<td>0.49±0.06</td>
<td>0.81±0.02</td>
<td>0.97±0.02</td>
<td>1.00±0.01</td>
<td>0.92±0.04</td>
<td>0.89±0.04</td>
<td>0.93±0.04</td>
</tr>
<tr>
<td>t-test</td>
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</tr>
<tr>
<td>WW</td>
<td>0.51±0.01</td>
<td>0.71±0.01</td>
<td>0.79±0.01</td>
<td>0.81±0.01</td>
<td>0.80±0.01</td>
<td>0.76±0.01</td>
<td>0.74±0.02</td>
</tr>
<tr>
<td>WS</td>
<td>0.48±0.05</td>
<td>0.70±0.01</td>
<td>0.80±0.01</td>
<td>0.81±0.01</td>
<td>0.77±0.02</td>
<td>0.75±0.03</td>
<td>0.77±0.02</td>
</tr>
<tr>
<td>t-test</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>MTVI2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td>NA</td>
<td>0.55±0.02</td>
<td>0.57±0.01</td>
<td>0.53±0.04</td>
<td>0.57±0.01</td>
<td>0.56±0.03</td>
<td>0.53±0.03</td>
</tr>
<tr>
<td>WS</td>
<td>NA</td>
<td>0.40±0.02</td>
<td>0.44±0.04</td>
<td>0.50±0.01</td>
<td>0.54±0.03</td>
<td>0.56±0.01</td>
<td>0.56±0.02</td>
</tr>
<tr>
<td>t-test</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

Data presented as mean ± SE. NA is no data, and different letters in a column represent significant difference at p ≤ 0.05. ns: not significant, *: significant at p ≤ 0.05, **: highly significant at p ≤ 0.01 using t-test.
of leaf expansion in cassava grown under water stress was significantly dropped (>50% reduction of control) within 6 d of water withholding and then quickly recovered in the re-watering process for 3 d (Alves and Setter, 2000). In addition, LAI is a key factor to regulate storage root yield of cassava (El-Sharkawy, 2007). Leaf relative water content in cassava cv. IAC 576-70 significantly declined depending on the days of water deficit stress especially after 90 d withholding period (Pereira et al., 2018) and genotypic dependence factor; for example, cv. ‘Nyalanda’ is sensitive to 10 d moisture stress (Turyagyenda et al., 2013).

Total chlorophyll content in the leaf tissues of water deficit stressed cassava cv. IAC 576-70 were maintained after water deficit treatments, leading to a constant maximum quantum yield of PSII (Fv/Fm) (Pereira et al., 2018). In contrast, Fv/Fm in cassava cv. SC5 was significantly

### Discussion

Leaf expansion (size), leaf retention, number of leaves and delayed senescence in cassava are directly related to growth and storage root yield, especially under the drought conditions (Alves and Setter, 2004; Turyagyenda et al., 2013; Liao et al., 2017). Number of new leaves and rate of plant height (cm day\(^{-1}\)) of cassava cv. C-1 under water deficit stress are significantly declined when compared with those in well-irrigated plants (Duque and Setter, 2013). In cassava cv. ‘CM 1585-13’, fallen leaves are evidently observed in the water deficit stressed plants and the emerged leaves are limited, leading to small leaf area and delayed shoot elongation (reduced height) when compared with control (well watering) (Calatayud et al., 2000). Leaf area and rate of leaf expansion in cassava grown under water stress was significantly dropped (>50% reduction of control) within 6 d of water withholding and then quickly recovered in the re-watering process for 3 d (Alves and Setter, 2000). In addition, LAI is a key factor to regulate storage root yield of cassava (El-Sharkawy, 2007). Leaf relative water content in cassava cv. IAC 576-70 significantly declined depending on the days of water deficit stress especially after 90 d withholding period (Pereira et al., 2018) and genotypic dependence factor; for example, cv. ‘Nyalanda’ is sensitive to 10 d moisture stress (Turyagyenda et al., 2013).

Total chlorophyll content in the leaf tissues of water deficit stressed cassava cv. IAC 576-70 were maintained after water deficit treatments, leading to a constant maximum quantum yield of PSII (Fv/Fm) (Pereira et al., 2018). In contrast, Fv/Fm in cassava cv. SC5 was significantly.

### Table 5. Calibrated and validated values of cassava VIs for estimated LAI under different water regimes

<table>
<thead>
<tr>
<th>Water regime</th>
<th>Input variable</th>
<th>Calibrated (n=21)</th>
<th>Validated (n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Model equation</td>
<td>R(^2)</td>
<td>RMSE</td>
</tr>
<tr>
<td>WW</td>
<td>NDVI</td>
<td>y = 0.0194e(^{5.8363x})</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>RVI</td>
<td>y = 0.7994e(^{10.922x})</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>EVI</td>
<td>y = 0.2322e(^{10.767x})</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>SAVI</td>
<td>y = 0.1000e(^{7.964x})</td>
<td>0.82</td>
</tr>
<tr>
<td></td>
<td>VARI</td>
<td>y = 1.2159e(^{216.56x})</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>MTVI2</td>
<td>y = 0.2753e(^{10.79x})</td>
<td>0.83</td>
</tr>
<tr>
<td>WS</td>
<td>NDVI</td>
<td>y = 0.0607e(^{537.7x})</td>
<td>0.71</td>
</tr>
<tr>
<td></td>
<td>RVI</td>
<td>y = 0.8193e(^{10.423x})</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>EVI</td>
<td>y = 0.3960e(^{-223.8x})</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>SAVI</td>
<td>y = 0.2218e(^{-41.01x})</td>
<td>0.69</td>
</tr>
<tr>
<td></td>
<td>VARI</td>
<td>y = 0.7802e(^{-229.9x})</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>MTVI2</td>
<td>y = 0.3919e(^{-229.9x})</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Fig. 6. Relationships between number of leaves per plant (120 DAP) and number of storage roots per plant (a), number of leaves per plant (150 DAP) and number of storage roots per plant (b), leaf area index (120 DAP) and storage root fresh weight (c) as well as leaf area index (150 DAP) and fresh storage root weight (d) of cassava grown under well-watered (WW) and water-stressed (WS) plots.
declined when exposed to water deficit for the long period (≥ 6d water withholding) (Liao et al., 2016; Liao et al., 2017). Reduction rate of Fv/Fm in cassava grown under water deficit stress was genotype-dependent; for example, Fv/Fm in cv. SC124 was more sensitive to water stress than that in cv. Arg7 (Zhao et al., 2015). In cassava cv. Cigana Preta, Fv/Fm in plants under nitrogen deficiency was maintained, whereas photon yield of PSII (ΦPSII) was diminished. On the other hand, non-photochemical quenching (NPQ) in nitrogen deficient plants was enhanced (Cruz et al., 2003). Under cold stress (5°C for 10 d), Fv/Fm and ΦPSII in two cultivars, SC8 and Col 1046 were significantly diminished, while NPQ was increased (An et al., 2016). In general, NPQ in the water deficit stressed plants was promoted with the high electron transport rate, which generates the heat toxicity at cellular levels (Chaves et al., 2002; Liu et al., 2008; Dahl and Vanlerberghe, 2018).

Biomass, number of storage roots and storage root yield per plant in cassava were sensitive to water deficit conditions (Adjepong-Danquah et al., 2016b; 2016c). In addition, the yield attributes of cassava under water shortage was genotype-dependent and can be rapidly recovered under re-watering process or precipitation (El-Sharkawy, 2007; Vandegeer et al., 2013). The planting date schedule relating to water shortage in weather forecasting is one of major factor to be concerned for cassava production using LINTUR model (Exzi et al., 2018).

The precipitation in the field directly affected the soil moisture in the WS and WW plots, and slightly different in the late growth stage. However, parameters in the early stage of cassava at 120 to 150 DAP (90 to 120 d water withholding) showed sensitivity to water deficit stress. The optimal LAI for storage root bulking was reported to be 3.0 to 3.5 m² m⁻² (Hillow et al., 2001), and the LAI for starch formation as reported in Thailand is 4.0 m² m⁻² (Boonseng, 2008). The number of leaves, leaf retention, and expanded size of leaves is peaked at 4 to 6 months after planting (MAP) and then leaf size and rate of leaf production are decreased at senescence (Ekanayake et al., 1998). Under water deficit stress, LAI was slightly reduced depending on the tolerant ability or genotype-dependent strategies to maintain the storage root yield (El-Sharkawy, 2004). Furthermore, this research demonstrated that LAI is a good indicator for overall growth performances and yield prediction in cassava. Therefore, storage root yield is declined when LAI exceeded (> 4 m² m⁻²), due to less carbohydrate assimilates in the storage roots (Ekanayake et al., 1998). Moreover, non-destructive vegetation models were validated. RVI was highly related to LAI of the cassava under different water regimes and can be applied to estimate LAI of cassava. The RVI to estimate over-story LAI, and normalized forms to the NDVI for reducing the impact of atmospheric scattering are good predictors of wet and dry green biomass, LAI and fractional cover (Thenkabail et al., 2012).

In cassava, a positive relationship between number of leaves per plant and number of storage roots in cassava genotypes has been demonstrated (R² = 0.44; p ≤ 0.001) in the Guinea Savannah Ecology of Ghana (Adjepong-Danquah et al., 2016a). Moreover, positive relation between number of leaves per plant and number of storage roots in cassava cv. MCol 1468 is validated with R² = 0.87 (Vandegeer et al., 2013). Storage root numbers in cassava are closely related with number of leaves (R² = 0.49; p ≤ 0.001) (Nawururuhunga et al., 2001). In contrast, independence between number of leaves and number of storage roots per plant was reported in both high cyanide cassava (R² = 0.22*) and low cyanide cassava (R² = 0.40**) in acid Ultisols of Southern Nigeria (Okpara et al., 2014a) as well as high cyanide cassava (R² = 0.22*) and low cyanide cassava (R² = 0.34**) in the humid tropics (Okpara et al., 2014b). In the water deficit stress, number of leaves in 20 elite clones of cassava was thoroughly related with the storage root yield (R² = 0.45; p ≤ 0.001) (Adjepong-Danquah et al., 2016b). For the yield traits, a positive relation between LAI and storage root yield has been widely established in cassava crop in Midwestern Nigeria (R² = 0.71; p ≤ 0.01; Odujgo, 2008), in tall- and short-stemmed rain-fed cassava cultivars (R² = 0.42; p ≤ 0.01; El-Sharkawy and de Tafur, 2010) and in 30 elite clones of cassava (R² = 0.42; p ≤ 0.001; El-Sharkawy et al., 2012). Recently, storage root yield in 20 cultivars of cassava grown under no irrigation increased in relation to the long harvesting times (12 MAP) (Adjepong-Danquah et al., 2016c).

Conclusions

Number of leaves and the LAI in cassava cv. ‘Huay Bong 80’ are good indicators to predict overall growth and storage root yield. Chlorophyll degradation and relative water content reduction in the leaf tissues are the key physiological indices in water shortage conditions of cassava. For non-destructive measurement model, the LAI as RVI should be applied to estimate cassava growth, biomass and storage root yield under water deficit conditions. The critical point at 150 DAP (120 d water withholding), the storage root bulking stage of cassava, is an indicator to growth development and yield performance of cassava. The LAI at 150 DAP was observed to estimate the storage root yield. The future LAI work may be validated under different environments and several dominating cassava cultivars within local genotypes. New remote sensing technologies, including very high spatial data, unmanned air vehicle and micro-hyperspectral imagery may further be applied in farmer field trials.

Acknowledgements

The authors would like to thank the Thai Tapioca Development Institute (TTDI), Nakhon Ratchasima province, Thailand, for the field trial validation. This research was supported by the National Research Council of Thailand (NRCT), the Cluster and Program Management Office (CPMO funding number P-13-00634) of the National Science and Technology Development Agency (NSTDA).
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Fig. Supplementary 1. NDVI (A), RVI (B), SAVI (C), EVI (D), VARI (E) and MTVI2 (F) vegetation indices in each time period after planting date

Fig. Supplementary 2. Relationships between NDVI and LAI (A), RVI and LAI (B), SAVI and LAI (C), and LAI EVI (D), VARI and LAI (E) and MTVI2 and LAI (F) in cassava under different water regimes
Fig. Supplementary 3. Calibration on NDVI (A), RVI (B), SAVI (C), EVI (D), VARI (E) and MTVI2 (F) vegetation indices using LAI parameter.