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EFFECTS OF DIFFERENT WATERING REGIMES ON FOLIAR SPECTRAL REFLECTANCE AND CHLOROPHYLL CONTENT OF *Jatropha curcas* Linn.

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ABSTRACT

The influence of high and reduced water availability on the changes in foliar spectral reflectance and chlorophyll of J. curcas were investigated. The field experiment conducted was based on a completely randomized design (CRD) with four treatments replicated three times. Each replicate consisted of two plants with a total of 24 plants altogether. Treatments were: (i) Rainfed (W0) – plants were watered at field capacity $\geq$ -0.03 MPa, (ii) Mild water stress (W1) – watering was carried out to maintain soil water potential between -0.30 and -0.10 MPa, (iii) Moderate water stress (W2) – watering was carried out to maintain soil water potential between -1.0 and -0.8 MPa, and (iv) extreme water stress (W3) – plants were grown at soil permanent wilt point $\leq$ -1.50 MPa. The well-watered plants responded by showing significantly ($p < 0.05$) better height growth. Result shows mean reflectance spectra increased with increasing water stress. As water stress indicator, foliar spectral analysis showed high reflectance which was primarily attributed to a 41 % reduction in chlorophyll concentration. The percentage reflectance at 550 (R_{550}), structure independent pigment index (SIPI), and photochemical reflectance index (PRI) showed a strong relationship with foliar chlorophyll content (SPAD). These results suggested that well-watered plants at soil water field capacity (-0.03 MPa) showed greener and healthier leaf growth compared to that of the water-stressed plants.

Keywords: *Jatropha curcas*, water stress, spectral reflectance, chlorophyll, soil water potential

INTRODUCTION

The *Jatropha curcas* shrub is commonly known as “Jarak Pagar” in Malaysia, is a member of the Euphorbiaceae family. It grows wild and is generally cultivated for the oil from its seeds, which is being used to produce bio-diesel

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fuel. *Jatropha curcas*'s potential for producing energy from marginal land without large input has previously created great attention, resulting in the planning of huge areas of plantation in Asia, Africa, and America (Oppenshaw 2000). Although *J. curcas* grows in semi-arid and arid tropical areas and can be considered as a drought tolerant species, several studies in Malaysia have shown that irrigated conditions and intensive cultivation gave better growth, hence, increased fruit and seed production as compared to rain-fed conditions (Zainudin et al. 2010). Spectral reflectance and chlorophyll fluorescence are rapid non-invasive methods that can be used to predict plant stress (Richardson & Berlyn 2002). Leaf reflectance is determined by the biochemical components of the leaf, including photosynthetic pigment concentrations – some of these which include chlorophylls, carotenoids and xantophylls are known to be affected by plant stress (Gitelson & Merzlyak 1996, Richardson et al. 2001). Typically, a reduction in leaf water content causes an increase in reflectance wavelengths from 500 to 700 nm (Carter 1994) attributed to the reduction of chlorophyll concentrations and desiccation in leaf structure at the cellular level (Carter 1994, Aldakheel & Danson 1997). In previous years, quantitative and rapid methods for evaluating leaf water status are important for plant water stress management in agriculture, horticulture, forestry, and fire risk management (Chandler et al. 1983, Inoue et al. 1993). Therefore, this study was conducted with the following objectives: (i) to investigate the foliar spectral reflectance of *J. curcas* exposed to different levels of water regimes, (ii) to quantify the relative chlorophyll content (SPAD) of *J. curcas* exposed to different levels of water regimes, and (iii) to study the relationship between spectral reflectance indices and leaf chlorophyll concentration as an indicator to plant water stress.

MATERIALS AND METHODS

A field study was conducted at a *J. curcas* farm situated near Universiti Malaysia Sarawak (UNIMAS) in Kota Samarahan, Sarawak, Malaysia. The 20 m x 29 m plot with relatively flat terrain was set up after which the experimental site was cleared and ploughed. Seeds were germinated in a sand bed at a germination rate of 80 %. After germination, the seedlings were transplanted into polythene bags with a mixture of 7:3:2 soil, sand, and peat. Uniform seedlings (in terms of height) were selected and transplanted at the planting site. Soil at planting site was the *Triboh* series which has a low to moderate permeability status, typical of a sandy clay loam (Soil Survey Staff 1992). The study was conducted from July 2009 to August 2009.

Experimental design and treatments

The experiment was a completely randomized design (CRD) with four treatments replicated three times. Each replicate consisted of two plants with a total of 24 plants altogether. Table 1 shows the different water stressed levels established within the experimental plots (International Resource Group 2006, Jongschaap et al. 2007). Water-proof canvas sheets (0.2 mm thick) with a surface area dimension of 4 m x 8 m covered the water stress plants whenever rain falls to mimic the periods when these species can tolerate drought in semi-arid and arid tropical areas (International Resource Group 2006, Jongschaap et al. 2007). In the case of accumulation of rain water on the canvas, a drainage system was constructed to channel water out from the water stressed plants. Treated plants were isolated by establishing small drains surrounding it to ensure the desired soil water potential (SWP). Metal sheets were inserted into the soil to a depth of 1.5 m surrounding the water stressed treatment to prevent lateral movement of water. Soil water potential was monitored by using a soil moisture sensor equipment (W.E.T. Sensor, Eijkelkamp, Netherlands).

Table 1: Levels of water regimes established by watering and withholding exposure to rainfall

Treatment	Description of soil exposure
W0	Entirely rain fed and watered all year round at FC $\geq$ -0.03 MPa
W1	Soil maintained at SWP between -0.30 and -0.10 MPa
W2	Soil maintained at SWP between -1.0 MPa and -0.8 MPa
W3	Soil maintained at permanent wilt point $\leq$ -1.5 MPa

W0 = control; W1 = mild water stress; W2 = moderate water stress; W3 = severe water stress; FC = field capacity; SWP = soil water potential

Plant height

Plant height measurement was obtained to verify the water stress effects. The plant's height from the base to the tip of the main stem was measured using a measuring tape.

Foliar spectral reflectance and chlorophyll content

The experiment was conducted from July 2009 to August 2009 with young fully expanded leaves of *J. curcas,* freshly collected in their natural state. A total of 24 leaves from six trees were taken from each treatment. In an attempt to minimize desiccation of cut branches, the samples were kept wrapped in a cool, moist paper towel inside a plastic bag. The samples were then placed inside a chest freezer filled with ice pending for reflectance measurements.

Spectral reflectance measurements at wavelengths from 400 to 2500 nm were done using the S.V.C. HR 1024 (Spectra Vista Corporation, New York, U.S.A.) with a standard 4^o Field of View (F.O.V.) at 1.0 m. Three indices were then derived from the percentage reflectance data sets to monitor changes in chlorophyll absorption: R_{550} which is the percentage reflectance at 550 nm (Moran et al. 2000), photochemical reflectance index [PRI = $(R_{531}-R_{570})/(R_{531}+R_{570})$] (Penueles et al. 1997), and structure independent pigment index [SIPI = $(R_{800}-R_{445})/(R_{800}-R_{680})$] (Peneules & Inoue 1999).

Relative chlorophyll content of leaves was determined by using chlorophyll meter (SPAD-502, Minolta, Japan). Readings were recorded when young fully expanded leaves with the same orientation and the same layer in the crown (middle bottom) were still attached to the tree.

Statistical analysis

Data were analyzed using one way analysis of variance (ANOVA) with the SPSS software (version 15). The Tukey's Honest Significance Difference (HSD) Test, at $\alpha = 0.05$ level of significance was done to compare the means and to determine whether there were any differences in the plant height and relative chlorophyll content between treatments. The relationships between spectral reflectance indices with relative chlorophyll content (SPAD) were tested by regression analysis of best fit.

RESULTS AND DISCUSSION

Plant height

The plant height of *J. curcas* planted under different levels of water stress from is presented in Table 2. The control plants recorded higher average value compared to that of the water-stressed plants. The plant height under W3 was about 18 % lower than the control at the end of the study period. The result indicates that water stress inhibited the growth of the plant's height as the soil water potential decreased from field capacity to $\geq$ -1.5 MPa. Jongschaap et al. (2007) documented that drought has profound effects on height which may well be a loss of turgor thus affects the rate of cell expansion and cell size.

Table 2: Effect of different water regimes on *J. curcas* plant height

Treatments	Plant height (cm)
W0	228.33 $\pm$ 0.45[a]
W1	217.17 $\pm$ 0.32[b]
W2	203.50 $\pm$ 0.93[c]
W3	188.00 $\pm$ 0.43[d]

Note: Plants were subjected to four different degrees of water stress (mean $\pm$ S.D., n = 24). Figures with same letter superscript within columns are not statistically different using Tukey's at P > 0.05 probability level. Treatments are W0 – control, W1 – mild water stress, W2 – moderate water stress, and W3 – severe water stress

Foliar spectral reflectance

The mean spectral reflectance for each treatment showed consistent increase in reflectance with increasing water stress at the visible wavelengths of 500 – 700 nm (Figure 1). Greater increase can be detected in the W3 treatment followed by W2, W1, and W0. The findings at the leaf cellular level by Penueles and Inoue (1999) reported that reflectance increased at all wavelengths with decreasing soil water potential. This is attributed to the decreased in leaf water content from fully turgid to dry state indicating plant exposure to water stress. In addition, Aldakheel and Danson (1997) hypothesized that increases in near infrared reflectance in response to foliage desiccation are related to changes in leaf structure at the cellular level. As water stress becomes severe, the cytoplasm shrinks and changes in light scattering at cell wall-water-air interfaces occur (Carter 1994). Meanwhile, the foliar spectral analysis illustrated how low reflectance (Figure 1) maybe attributed to absorption by higher concentration of chlorophyll photosynthetic pigments and cytoplasmic fluid and is similar to the finding by Carter (1994).

Comparisons between control and water stressed treatment of the leaf relative chlorophyll content (SPAD) are presented in Table 3. The treatment mean for W0 was the highest with a SPAD value of 49.13 followed by W1, W2, and W3 with SPAD values of 41.13, 36.73, and 29.25 respectively. The results showed that the chlorophyll concentration in plants grown under water stressed conditions was significantly ($p < 0.05$) reduced compared to those in the control (Table 3). Chlorophyll content was reduced by 41 % as the soil water potential decreased from field capacity to $\leqq$ -1.5 MPa, indicating that water stress depressed leaf chlorophyll concentration of *J. curcas* substantially. The majority of chlorophyll lost from leaves subjected to drought stress is lost from the mesophyll cells (Richardson & Berlyn 2001).

The reasons for this preferential loss could be attributed to the fact that the mesophyll cells are farther removed from the vascular supply of water, and hence develop greater cellular water deficits which lead to a greater loss of chlorophyll. In the absence of sufficient cytoplasmic fluid, there was a slow breakdown of chlorophyll photosynthetic pigments (Carter 1994).

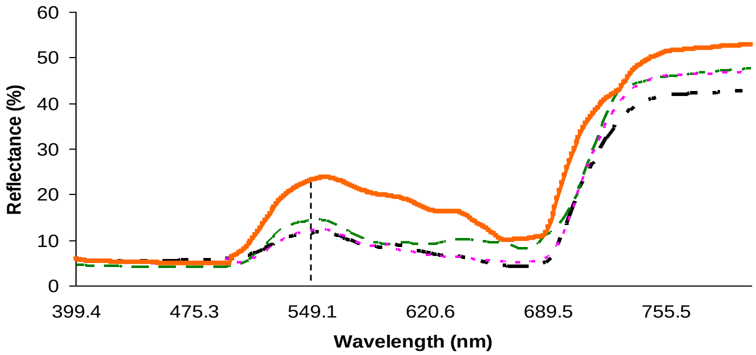

Figure 1: Mean laboratory spectral reflectance of *J. curcas* leaves under different water regimes at wavelengths ranging from 400 – 800 nm. Treatments are W0 – control (■), W1 – mild water stress (■), W2 – moderate water stress (■), and W3 – severe water stress (■)

Table 3: Effect of different water regimes on *J. curcas* leaf_relative chlorophyll content (SPAD)

Treatments	Chlorophyll (SPAD)
W0	49.13 $\pm$ 0.91[a]
W1	41.13 $\pm$ 1.10[b]
W2	36.73 $\pm$ 0.43[c]
W3	29.25 $\pm$ 0.49[d]

Note: Plants were subjected to four different degrees of water stress (mean $\pm$ S.D., n = 24). Figures with same letter superscript within columns are not statistically different using Tukey's at P > 0.05 probability level. Treatments are W0 – control, W1 – mild water stress, W2 – moderate water stress, and W3 – severe water stress

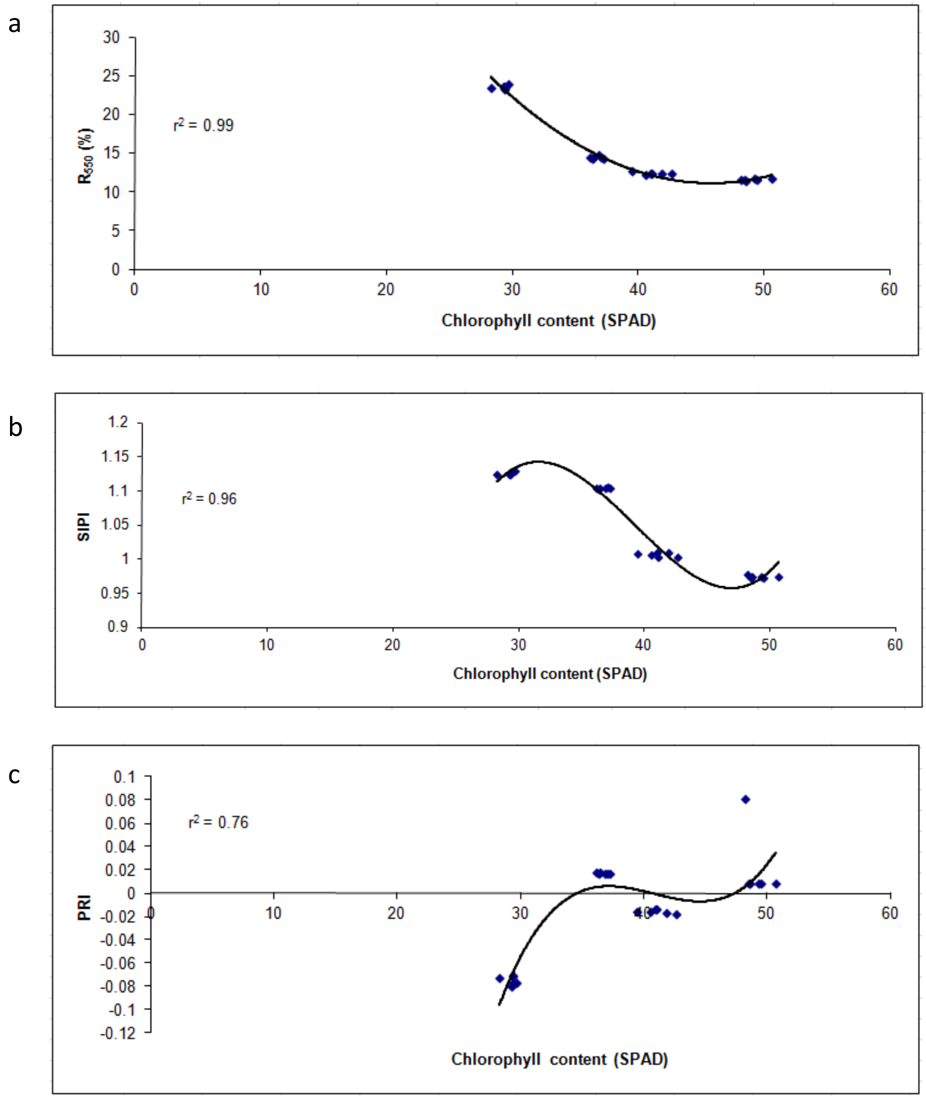

Figure 2: Relationship between several reflectance indices (R_{550}, SIPI, and PRI) and relative chlorophyll content (SPAD) in *Jatropha curcas* leaves subjected to water stress. Values are means ± s.e. of twenty four leaves taken from different plants per treatment. The regression lines (continuous) are shown. The values of the determination coefficient are included

Values of R_{550} decreased as foliar chlorophyll concentration increased while the relationship was best described by a polynomial cubic regression (Figure 2a). Correlation coefficient value of $r^2 = 0.99$ indicating a potent relationship between the R_{550} wavelength and leaves chlorophyll concentration (Table 4).

The SIPI values also decreased with increasing chlorophyll concentration as portrayed by a polynomial inverse third-order regression in Figure 2b. The analysis shows a strong correspondence between SIPI and leaf chlorophyll concentration with r^2 = 0.96. The outcome indicates that SIPI is also a good predictor for leaf chlorophyll concentration (Table 4). In addition, the PRI increased with increasing chlorophyll concentration (Figure 2c), and the relationship was best described by a polynomial inverse third-order regression. A correlation coefficient value of 76 % of the variation in PRI suggested that both R_{550} and SIPI can be regarded as a better predictor of chlorophyll concentration than PRI (Table 4).

Table 4: Regression equations for reflectance measurements versus foliar relative chlorophyll content of *J. curcas* leaves. The data are plotted in Figure 2

Reflectance index	**Regression equation**	**r^2**	**n**
R_{550}	y = 0.0446 $(Chl)^2$ - 4.0912(Chl) + 104.83	0.99	24
SIPI	y = 0.0001$(Chl)^3$ - 0.012x2 + 0.4533(Chl) - 4.4111	0.96	24
PRI	y = 6E-05$(Chl)^3$ - 0.0079$(Chl)^2$ + 0.322(Chl) - 4.3164	0.76	24

The values of R_{550} derived from spectral reflectance measurements were strongly correlated (r^2 > 0.99) with the chlorophyll content from the control and water stressed plants. This relationship suggests that R_{550} can be a reasonable indicator of medium to high chlorophyll concentrations (Moran et al. 2000). Similar non-linear correlation was found also between reflectance index SIPI and chlorophyll content of leaves probably because two of the wavelengths used in the SIPI derivation (445 nm and 680 nm) fall within the waveband associated with absorbance by chlorophylls (Penueles & Inoue 1999). However, a lower correlation value was obtained when PRI was correlated with chlorophyll concentration values (r^2 > 0.76). The photochemical reflectance index (PRI) is based on the fact that changes in the xanthophyll cycle can be detected by monitoring reflectance at 531 nm compared with an insensitive reference wavelength at 570 nm (Penueles et al. 1997). These correlations concurred with the previous work by Adams et al. (1999) which reported that further evidence of chlorophyll degradation is the broadening of the reflectance peak around 550 nm, observed as slight leaf yellowing.

Because foliar concentrations of photosynthetic pigments, most notably the chlorophylls, are affected by a variety of stress factors, reflectance analysis can be an early indicator to provide a means of assessing the degree to which plants are affected by water stress. In this study, with soil water at field capacity (-0.03 MPa), well watered plants generally showed healthier leaves growth than that of the water stressed plants. It is worth of a mention at this time that the positive effects on the well-watered plants leaf growth can be very favourable to the plant's photosynthesis.

CONCLUSION

In well-watered (W0) plants, the high availability of soil water at field capacity (-0.03 MPa) showed profoundly better height than that of the water-stressed plants. This study has shown that damage caused by drought at leaf cellular level increased the foliar reflectance wavelengths. Foliar spectral analysis showed high absorbance when there was an absorption by higher concentration of chlorophyll photosynthetic pigments and the presence of cytoplasmic fluid at the leaf cellular level. Strong significant correlations were achieved by the reflectance indices such as R_{550}, SIPI, and PRI indicating the depression caused by water stress to chlorophyll concentrations in leaves. Results from foliar spectral reflectance can be a good indicator for assessing the degree of water stress at an early stage. Overall, W0, which was the rain fed treatment, grew significantly better than that of the water stressed plants thus indicating that with the availability of soil water at field capacity (-0.03 MPa), *J. curcas* can adapt successfully to the hot and humid climatic conditions of Malaysia.

REFERENCES

Adams, M.L., Philpot, W.D., Norvell, W.A. 1999. Yellowness index: An application of spectral second derivatives to estimate chlorosis of leaves in stressed vegetation. *International Journal of Remote Sensing* **20**: 3663–3675.

Aldakheel, Y.Y., Danson, F.M. 1997. Spectral reflectance of dehydrating leaves: measurements and modelling. *International Journal of Remote Sensing* **18**: 3683–3690.

Carter, G.A. 1994. Ratios of leaf reflectances in narrow wavebands as indicators of plant stress. *International Journal of Remote Sensing* **15**: 697–703.

Chandler, C., Cheney, P., Thomas, P., Trabaud, L., Williams, D. 1983. Forest fire behaviour and effects. In: *Fire in Forestry (Vol 1)*. J. Wiley & Sons, New York, 45-46.

Gitelson, A.A., Merzlyak, M.N. 1996. Signature analysis of leaf reflectance spectra: Algorithm development for remote sensing of chlorophyll. *Plant Physiology* **148**: 494–500.

Inoue, Y., Morinaga, S., Shibayama, M. 1993. Non-destructive estimation of water status of intact crop leaves based on spectral reflectance measurements. *Japan Journal of Crop Science* **62**: 462–469.

International Resource Group. 2006. Irrigation and crop management plan. Report no. 25. Published by United States Agency for International Development, 25.

Jongschaap, R.E.E., Corre, W.J., Bindraban, P.S., Brandenburg, W.A. 2007. Claims and Facts on *Jatropha curcas* L. Report no. 158. Global *Jatropha curcas* evaluation, breeding, and propagation programme. Published by Plant Research International. Wageningen, 6.

Moran, J.A., Mitchell, A.K., Goodmanson, G., Stockburger, K.A. 2000. Differentian among effects of nitrogen fertilization treatments on conifer seedlings by foliar reflectance: a comparison of methods. *Tree Physiology* **20**: 1113–1120.

Oppenshaw, K. 2000. A review of *Jatropha curcas*: An oil plant of unfulfilled promise. *Biomass and Bioenergy* **19:** 1–15.

Penueles, J. and Inoue, Y. 1999. Reflectance indices indicative of changes in water and pigment contents of peanut and wheat leaves. *Photosynthetica* **36** (3): 355–360.

Penueles, J., Pinol, J., Ogaya, R. and Filella, I. 1997. Estimation of plant water content by the reflectance water index WI (R900/R970). *International Journal of Remote Sensing* **18**: 2869–2870.

Richardson, A.D. and Berlyn, G.P. 2002. Changes in foliar spectral reflectance and chlorophyll fluorescence of four temperate species following branch cutting. *Tree Physiology* **22**: 499–506.

Richardson, A.D., Berlyn, G.P. and Gregoire, T.G. 2001. Spectral reflectance of *Picea rubens* (Pinaceae) and *Abies balsamea* (Pinaceae) needles along an elevational gradient, Mt. Moosilauke, New Hampshire, USA. *American Journal of Botany* **88**: 667–676.

Soil Survey Staff. 1992. Keys to Soil Taxonomy. 5th Eds. In: SMSS Technical Monograph No. 19. Blacksberg, Virginia, USA.

Zainudin, S.R., Muyang, K., Mustafa, K., and Ismawi, Z. 2010. Photosynthetic and stomatal responses of *J. Curcas* to different watering regimes. *Proceedings of the 11th Symposium for the Malaysian Society of Applied Biology 2010*, 406–413.