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FOLIAR ABSCISIC ACID (ABA) CONCENTRATION AND LEAF GAS EXCHANGE PROPERTIES OF *JATROPHA CURCAS* SUBJECTED TO WATER STRESS

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ABSTRACT

International interest in Jatropha curcas as a drought tolerant, fast-growing crop, suitable for bio-energy production, has grown significantly in recent years. The effective management of commercially planted species in terms of possible water resource impacts requires accurate information on water use and bio-physical production characteristics relevant to areas having planting potential. Currently there is no knowledge on the type of water regimes suitable for the survival, growth and yield performances of J. curcas particularly in Sarawak which seems to be receiving rainfall all year round. Foliar abscisic acid (ABA) concentration and leaf gas exchange properties of Jatropha curcas subjected to water stress were examined. Foliar ABA concentration of J. curcas increased to 5 fold while its leaf stomatal conductance (g_s) was reduced by 31 % as the soil water potential decreased from field capacity to more than 1.5 MPa. Photosynthetic rates (A) of plants grown under control conditions were higher with mean values ranging from 13.69 to 20.27 μmol m^{-2} s^{-1} compared to those under water-stress with mean values ranging from 8.39 to 15.47 μmol m^{-2} s^{-1}. Photosynthetic rates were however reduced by 41 to 39 % from April to July after as the soil water potential decreased from field capacity to > 1.5 MPa indicating that water stress depressed photosynthetic capacity of J. curcas. A strong relationship between A and g_s regardless of treatments (r^2 = 0.86) was observed indicating that photosynthesis was closely related to the changes in the leaf stomatal conductance and overall plant growth performance was closely correlated to photosynthetic rates. Under water stress, the fivefold increment of foliar ABA assisted plant in adaptation to drought stress through stomata closure thus reducing excessive transpiration. The combinations of physiological mechanisms that effectively postpone dehydration and minimize damage enable this species to survive in hostile environment with unpredictable precipitation.

Keywords: *J. curcas*, water availability, photosynthesis, stomatal conductance, growth performances

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INTRODUCTION

Jathropa curcas (J. Curcas) can be an alternative for biodiesel production due to its main trait as a renewable and sustainable fuel. an economically viable alternative species to replace fast-depleting fossil fuels and also due to its adaptability to marginal soils and environments (Dehgan & Webster 1979). The plant is a multipurpose shrub found throughout the tropics, known by 200 different names, and is a native of South America, but also widely cultivated throughout Central America, Africa and Asia and world-wide (Dehgan & Webster 1979, Oppenshaw 2000). This highly drought-resistant species is adapted to arid and semi-arid conditions (Oppenshaw 2000). The current distribution shows that growth has been most successful in the drier regions of the tropics with annual rainfall of 300-1000 mm. It grows mainly at lower altitudes (0-500 m) in areas with average annual temperatures well above 20 °C but can grow at higher altitudes and tolerates slight frost. It grows on well-drained soils with good aeration and it is claimed to be well adapted to marginal soils with low nutrient content (Oppenshaw 2000).

Research on growth performance of *J. curcas* was mainly focused on its suitability to dry and arid lands. The effective management of commercially planted species in terms of possible water resource impacts requires accurate information on water use and bio-physical production characteristics relevant to areas having planting potential. Currently there is no knowledge on the type of water regimes suitable for the survival, growth and yield performances of *J. curcas* particularly in Sarawak which seems to be receiving rainfall all year round. Thus, the objective of this study was to determine whether the crop can adapt successfully to the hot and humid climatic conditions of Sarawak. Therefore, this study were conducted with the following objective: (i) To investigate the impact of limited and excessive water availability on various physiological traits, and (ii) to examine the possible role of ABA in mediating the impact of high and reduced water availability.

MATERIALS AND METHODS

The study was conducted at *J. curcas* farm situated near Universiti Malaysia Sarawak (UNIMAS) in Kota Samarahan, Sarawak, Malaysia at longitude and latitude of 01^{o}24' N and 110 ^{o}24' E. Mature seeds of *J. curcas* were collected from Lundu, Sarawak. The seeds were germinated in a sand bed. After germination, the seedlings were transplanted into polythene bags with a mixture of 7:3:2 (v/v) soil, sand and peat. After 5 months, uniform seedlings with uniform height of 0.5 m were selected and transplanted at the planting site. The soil at the planting site was of the *Triboh series* (Soil Survey Staff 1992). The duration of the study was from April 2009 to October 2009.

Experimental design and treatments

The experiment was a complete randomized design (CRD) with four treatments replicated 3 times. Each replicate consisted of two plants with a total of 24 plants. Table 1 shows the different water stress levels established within the experimental blocks by manipulating the level of exposure to rainfall. Canvas sheets (0.2 mm thick) with a surface area dimension of 4 m x 8 m, covered the water stressed plants whenever rain falls to mimic the dry periods when these species can tolerate drought in semi-arid and arid tropical areas (Jongschaap et al. 2007). In case of accumulation of rain water on the canvas for water stressed plants, a drainage system was constructed to channel water out from the water stress plants. Metal sheets were inserted into the soil to a depth of 1.5 m surrounding the water stress treatment to prevent lateral movement of water. Rainfall was restraint by covering this treatment with canvas sheets at every 3 weeks interval.

Gas Exchange Measurements

From the month of April to October 2009, onsite preliminary diurnal gas exchange measurements were carried out on young fully expanded leaves aged approximately 7 months old with the same orientation and the same layer in the crown (middle bottom). Measurements of net photosynthesis on an area basis (A) (μmol CO_2 $m^{-2}s^{-1}$), leaf stomatal conductance (g_s) (mol H_2O $m^{-2}s^{-1}$), and transpiration rate (E) (mmol H_2O $m\text{-}^{2}s^{-1}$) of fifteen different leaves per treatment were monitored using a LICOR 6400 (Lincoln, Nebraska, USA) infrared gas analyzer (IRGA). Light intensity (Photosynthetically Active Radiation, PAR) within the sampling chamber was set at 1500 μmol $m^{-2}s^{-1}$, using a Li-6400-02B LED light source (LI-COR). The CO_2 flow into the chamber was maintained at a concentration of 400 μmol mol^{-1} using a LI-6400-01 CO_2 mixer (LI-COR). The humidity flow into the chamber was fixed at 500 μmol s^{-1}, and desiccant mid-range between scrub and bypass. To assess the trade-off between CO_2 uptake and water loss, instantaneous water-use efficiency (WUE) was calculated as ratio between photosynthetic rate and transpiration rate (A/E, μmol CO^2/μmol H_2O). Regression lines of best fit were applied to the monthly data relating A to g_s and g_s to volumetric soil water (%). Volumetric soil water content was measured using a soil moisture sensor equipment (W.E.T. Sensor, Eijkelkamp, Wageningen, Netherlands). Gas exchange parameters were taken at between 1100 to 1200 h, which was presumed to be the diurnal period when photosynthetic rates would be maximal (DiCristina & Germino 2006).

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

Treatment	Description of soil exposure
W0	Rain fed and watered throughout the year at FC $\geq$ -0.03 MPa
W1	Soil maintained at SWP $\geq$ -0.30 MPa
W2	Soil maintained at SWP $\geq$ -1.0 MPa
W3	Soil maintained at SWP $\geq$ -1.5 MPa

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

Foliar ABA Concentration Measurement

The analysis of foliar ABA concentration was determined according to the method by Kozukue et al. (1984). Leaves were collected and wrapped with aluminium foil, immediately dipped in liquid nitrogen and kept at -20 ^{o}C in the freezer. Prior to methanol (MeOH)-water preparation, the MeOH was kept cold in a refrigerator until used. Cold MeOH-water (200 mL) was prepared by mixing both 160 mL of MeOH and 40 mL of distilled water together. Dried leaves (10 g) were grounded with the cold MeOH-water. The extract was filtered and the solid materials were again grounded with cold MeOH-water and filtered. The two filtrates were combined, and the MeOH was removed using the rotor evaporator. All crude extracts was analyzed using silica thin layer chromatography (TLC) before performing column chromatography (CC) and as well as during CC to monitor the fractions obtained. The solvent system for these purposes was ethyl acetate. The TLC was visualized using UV light at 254 nm, vanillin dipping, and iodine vapour test.

Each crude extract was then dissolved in 100 mL of 0.05 M phosphate buffer (pH 8.0), washed three times with 50 mL of petroleum ether (PE) and the upper phase (PE) was discarded. The lower phase (phosphate buffer) was adjusted to pH 2.8, and extracted (50 mL x 4) with ethyl acetate. The combined ethyl acetate phases were removed using rotor-evaporator and the extract was dissolved in 4 mL of 0.05 M phosphate buffer (pH 8.0) readied for CC. The CC solid phase was made from Sephadex G-10 gel (31.80 g) which has been made into slurry by adding 25 mL of 0.05 M phosphate buffer (pH 8.0). Before pouring into the column (50 x 1.4 cm), the column was first plugged with small piece of cotton wool and 10 mL 0.05 M phosphate buffer

solvent was then added. A spatula of acidified sand was added to make about 0.5 cm height of sand layer above the cotton wool. Solvent was further added to about 1/3 of the column followed by Sephadex slurry with a few rinsing of 0.05 M phosphate buffer. Sephadex gel in the column was compacted for a 4 days to ensure all silica had a uniform distribution of adsorbent.

Each 4 mL aliquot from all extracts was chromatographed separately using CC previously equilibrated with 0.05 M phosphate buffer (pH 8.0), and the components were eluted with the same buffer. The flow-rate was 0.33 mL/mm. The eluate was collected in 5-mL fractions and each fraction was monitored using TLC. To identify compound ABA, the TLC of fractions were compared with TLC with that of the standard ABA concentration. Each partially purified plant extract containing ABA from these fractions was collected, adjusted to pH 2.8 and then extracted with ethyl acetate (30 mL x 5) and the combined ethyl acetate (lower phase) was removed using rotary evaporator**.** A 500 µL volume of MeOH (HPLC grade) was added to the residue in preparation for HPLC.

A 20 µL of the solution as obtained above were then injected into a Lichrospher 100 RP-18 E column and eluted with a linear gradient of 10 to 50% acetonitrile in 1% glacial acetic acid in water. Operating conditions were: UV detection at 254 nm; flow-rate, 1 ml/min; column temperature, 55^{o}C; gradient profile was initially 10 % acetonitrile and 90 % (1 % glacial acetic acid in water) for 3 minutes, 20 % acetonitrile and 80 % (1 % glacial acetic acid in water) for 3 minutes, 30 % acetonitrile and 70 % (1 % glacial acetic acid in water) for 3 minutes, 40 % acetonitrile and 60 % (1 % glacial acetic acid in water) for 3 minutes, and finally 50 % acetonitrile and 50 % (1 % glacial acetic acid in water) for 3 minutes; column was re-equilibrated for 5 minutes with mobile phase solvents; chart speed, 2.5 mm/min. The compound identification of ABA was performed by the co-injection method. Volume of 20 µl of authentic ABA (5 mg per 2.5 mL MeOH) with a concentration of 2.0 mg/L was mixed together with each extract and the solution was injected into the Lichrospher 100 RP-18 E column. Comparison of retention times of the peaks in authentic hormone and in the purified plant extracts were obtained by HPLC. Quantification of ABA was accomplished by comparing their peak areas in samples to the peak area of the standard.

Quantification of ABA in ng/g form was calculated using the formula:
Example:
Sample ABA concentration (ng/g)

$$= \left[\frac{\text{Conc. of standard from calibration curve (mg/L)} - \text{Peak area sample}}{\text{Peak area of standard from calibration curve}}\right] \times \left(\frac{1000000}{(1000)}\right)$$

DATA ANALYSIS

Data were analyzed using one way analysis of variance (ANOVA) with the SPSS software (version 15, SPSS Inc., Chicago, USA). 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 among the physiological parameters between treatments. Gas exchange parameters obtained were expressed as mean $\pm$ S.E. from measurements of fifteen leaves per treatment.

The relationship of $A - g_s$ and g_s – volumetric soil water content (%) were tested by regression analysis, using midday gas measurements averages over the study period as experimental units. Foliar ABA concentration – leaf stomatal conductance and that of foliar ABA concentration – volumetric soil water content was correlated using regression of best fit.

RESULTS AND DISCUSSION

Foliar ABA concentration and stomatal conductance

The foliar ABA concentration of plants in W3 treatment increased by 5 fold as the soil water potential decreased from field capacity to $\geq$ -1.5 MPa (Table 2). The leaf stomatal conductance for W3 was reduced by 31 % as the soil water potential decreased to $\geq$ -1.5 MPa. The result indicates that the increase in foliar ABA concentration was associated with the decline in stomatal conductance of water-stressed plants. Similarly, a strong relationship (r^2=0.96) between leaf stomatal conductance and foliar ABA concentration of *J. curcas* exposed to different levels of water stress in which leaf stomatal conductance increased with decreasing foliar ABA concentration (Figure 1).

Table 2: Foliar ABA concentration and leaf stomatal conductance of *J. curcas* under different water regimes.

Treatments	**Foliar ABA concentration (ng/g)**	**Leaf Stomatal Conductance (mol H_2O m^{-2} s^{-1})**
W0	523.00 ± 3.35^{a}	0.13 ± 0.01^{a}
W1	546.00 ± 3.58^{b}	0.12 ± 0.03^{b}
W2	885.33 ± 3.01^{c}	0.10 ± 0.02^{c}
W3	2437.33 ± 4.60^{d}	0.08 ± 0.03^{d}

Note: Figures with same letter superscript within columns are not statistically different using Tukey's at $P < 0.05$

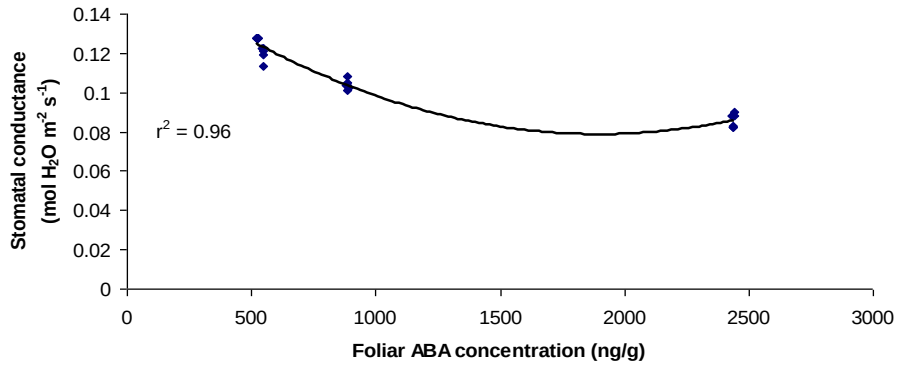

Figure 1: Relationship between leaf stomatal conductance and foliar ABA concentration in *J. curcas* subjected to water stress. Values are means of five leaves taken from different plants per treatment.

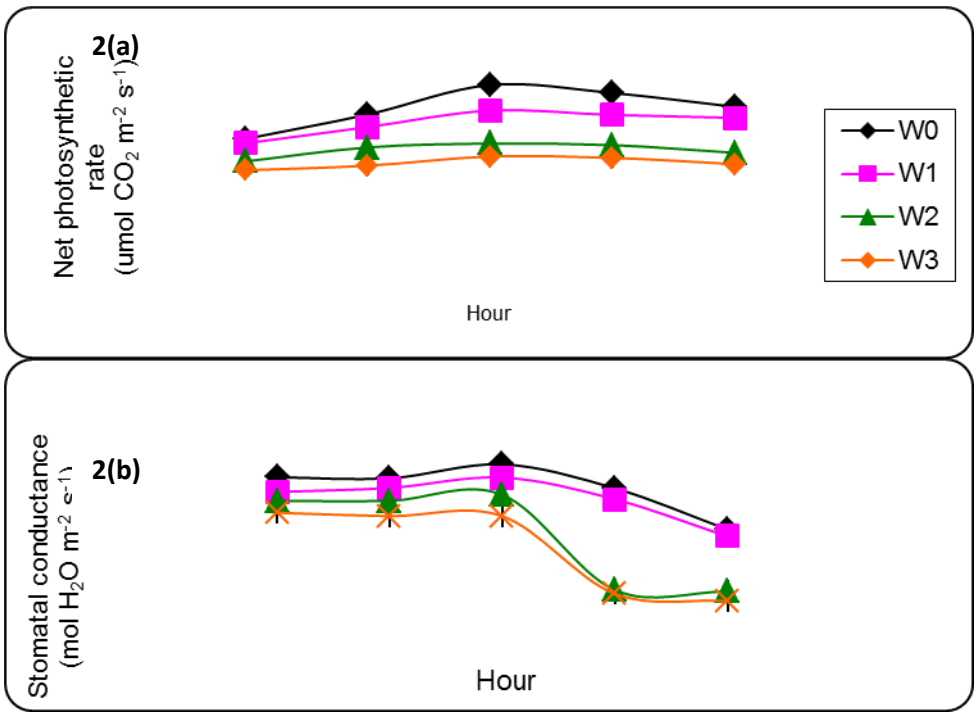

Figure 2(a) and 2(b): Diurnal changes of mean leaf photosynthesis (A) and stomatal conductance (g_s) to water vapour in *J. curcas* under different levels of water regimes. Values are means of fifteen leaves taken from different plants per treatment. Treatments are as in Table 1.

The diurnal mean leaf photosynthesis rate (*A*) of *J. curcas* under different levels of water regimes are shown in Figure 2(a). Plants grown under control conditions gave higher values ranging from 11.10 – 16.57 μmol CO_2 m^{-2} s^{-1} compared to those under water-stressed with values of 7.92 – 9.32 μmol CO_2 m^{-2} s^{-1}. Regardless of treatments, *A* values were observed highest during the 1200 hour and can be attributed to the considerable availability of Photosynthetic Active Radiation (PAR) throughout the study period. From noon, all *A* values declined slightly towards the 1600 hour in which could be due to either higher evaporative demand or the reduction of PAR. There were significant ($p < 0.05$) differences among all treatments in leaf *A* as affected by water stress in the months of April, July, and October 2009 (Table 3). Photosynthetic rates were reduced by about 41, 39, and 51 % in the month of April, July, and October respectively as the soil water potential decreased from field capacity to ≥ 1.5 MPa indicating that water stress substantially depressed photosynthetic capability of *J. curcas* plants (Table 3).

Leaf stomatal conductance (g_s)

All plants under rain fed conditions gave higher g_s than those in water-stressed plants with values ranging from 0.09 – 0.14 mol H_2O m^{-2} s^{-1} (Figure 2b). Regardless of treatments, the g_s values were relatively higher at the 1200 hour which contributed to higher *A* of the plants. This was due to the impact of stomatal opening which maintained photosynthetic efficiency without much considerable change in water potential. There were significant ($p < 0.05$) differences among all treatments in g_s as affected by water stress in the months of April, July, and October 2009 (Table 3). At the end of the experiment, g_s of W3 was reduced by 29 % of the control as the soil water potential decreased from field capacity > 1.5 MPa. Similarly, a strong relationship (r^2=0.86) between leaf photosynthesis rate (*A*) and leaf stomatal conductance (g_s) of *J. curcas* exposed to different levels of water stress indicating that photosynthesis rate was therefore, closely related to the changes in the leaf stomatal conductance (Figure 3).

DISCUSSION

The photosynthesis rates (*A*) recorded in response to water stress were consistent with that of Cornic and Massacci (1996) in which water stress decreased the rate of CO_2 assimilation per unit leaf area with constant and diurnal photosynthetic active radiation (PAR) and independent of the CO_2 supply outside the leaf. The decreased can be attributed to the direct inhibition of biochemical processes caused by increased in abscisic acid (ABA) and were induced by loss of cellular water. Some other factor that contributed to the decreased in *A* might be the limited CO_2 diffusion into the intercellular spaces of the leaf as a consequence of reduced stomatal conductance (Lawlor 2002). The result was parallel to that of Schulze and

Table 3: Photosynthesis (*A*) and Stomatal Conductance (g_s) measurements of *J. curcas* under different water regimes in April, July, and October 2009.

Month	Treatments	Photosynthesis (*A*) (µmol CO_2 $m^{-2}s^{-1}$)	Stomatal Conductance (*gs*) (mol H_2O m^{-2} s^{-1})
	W0	15.74 ± 0.05[a]	0.15 ± 0.03[a]
April	**W1**	13.18 ± 0.08[b]	0.14 ± 0.02[b]
	W2	9.70 ± 0.07[c]	0.12 ± 0.03[c]
	W3	9.24 ± 0.10[d]	0.10 ± 0.03[d]
	W0	13.69 ± 0.10[a]	0.13 ± 0.01[a]
July	**W1**	13.26 ± 0.03[b]	0.12 ± 0.03[b]
	W2	10.38 ± 0.10[c]	0.10 ± 0.02[c]
	W3	8.39 ± 0.06[d]	0.09 ± 0.03[d]
	W0	20.27 ± 0.22[a]	0.14 ± 0.03[a]
October	**W1**	15.47 ± 0.26[b]	0.13 ± 0.02[b]
	W2	11.32 ± 0.14[c]	0.12 ± 0.02[c]
	W3	9.90 ± 0.07[d]	0.10 ± 0.02[d]

Note: Figures with same letter superscript within columns are not statistically different using Tukey's at $P > 0.05$

Hall (1982) where tree species from the deserts and temperate regions closed its stomata as the soil water potential decreased rapidly. The ability to respond directly to drought provided a means by which a reduction in water loss and the production of tissue water deficits can be avoided. When plants were exposed to water stress, *A* and *gs* generally decreased to a limited water supply, implicated the involvement of a root signal that was induced as soil water potential falls (Gollan et al. 1986). Zainudin and Awang (2004) reported that when plants were under water stress, it stimulates the production of ABA in roots which travels via the xylem to the leaf where it decreases stomatal conductance. When roots sensed dryness, the root hormones may decrease leaf stomatal conductance and transpiration before any leaf water potential changes occur in leaves and shoots. Stomata regulate transpiration to allow sufficient carbon gain while preventing leaf water potential from becoming too negative (Kang and Zhang 2004). Effective control is therefore important for plant growth and survival especially when water supply is limited.

J. curcas plantations have traditionally been designed using low input with minimal consideration given to water requirements. The plant can grow on any kind of soil even in marginal areas; low yields have been recorded in low rainfall areas while it produces higher yields in higher rainfall or irrigated areas. In India, only recently has water availability been a goal of plantation design due to lengthy droughts, depletion of water tables, water rationing and poor water quality which has significant negative impact on both yield and quality of fruits. Although the crop has a reputation as a drought tolerant plant, *Jatropha* growers in Malaysia should look into appropriate irrigation technique to maintain the soil water at its field capacity (-0.3 MPa) during period of prolonged drought. Optionally, specific water stress (SWP > -1.5 MPa) at a short period of time during the plant's initial stages of development could result in desirable improvements to its stress tolerance. Probably supplementing irrigation plus organic fertilization may help to improve the growth and development of the plant in Sarawak. Future studies may consider growing it on different types of soils in Sarawak especially on peat.

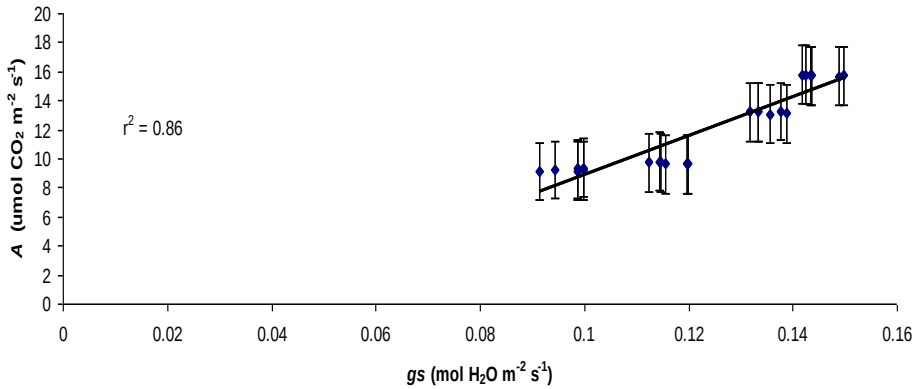

Figure 3: Relationship between leaf photosynthesis rate (*A*) and stomatal conductance (g_s) in *J. curcas* subjected to different levels of water regimes. Values are means of fifteen leaves taken from different plants per treatment

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CONCLUSIONS

Foliar ABA concentration of *J. curcas* under water potential of $\geq$ 1.5 MPa (W3) increased to 5 fold while its leaf stomatal conductance was reduced by 31 % as the soil water potential decreased from field capacity to $\geq$ 1.5 MPa. Photosynthesis rates of plants grown under control conditions were higher with mean values ranging from 13.69 to 20.27 µmol m-^{2} s^{-1} compared to those under water-stressed with mean values of 8.39 to 15.47 µmol m^{-2} s$^{-1.}$

Photosynthesis rates were however, reduced by 41, 39, and 51 % in the month of April, July, and October in plants subjected to SWP at permanent wilting point indicating that water stress depressed photosynthetic capacity of *J. curcas*. A strong relationship between *A* and g_s (r^2 = 0.86) was observed indicating that photosynthesis was closely related to the changes in the leaf stomatal conductance and overall plant growth performance was closely correlated to photosynthetic rates. Under water stress, the 5 fold increment of foliar ABA had assisted plant in adaptation to drought stress through stomata closure thus reducing excessive transpiration. The combinations of physiological mechanisms that effectively postpone dehydration and minimize damage enable this species to survive in hostile environment with unpredictable precipitation.

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