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Effect of Water Deficit Stress on Photosynthetic Characteristics of *Jatropha curcas*

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Abstract

The need to mitigate climate change cannot be more emphasized, which arises, as a result of increases in CO_2 emissions due to anthropogenic activities. Given the current world energy problems of high fossil fuel consumption which plays a pivotal role in the greenhouse effect, *Jatropha curcas* biodiesel has been considered a potential alternative source of clean energy (biodiesel is carbon neutral). However, the ability of *Jatropha curcas*, as a candidate source of alternative of clean energy, to grow in marginal and dry soils, has been poorly elucidated. This study, therefore aimed at investigating whether *Jatropha curcas* leaves could switch from carrying out C_3 photosynthetic pathway to Crassulacean Acid Metabolism (CAM) as a strategy to improve its water deficit tolerance. Thirty-five-day-old *Jatropha curcas* accessions, from three different climatic zones of Botswana, *viz.*, Mmadinare (Central zone), Thamaga (Southern zone) and Maun (Northern zone), were subjected to water stress, by withholding irrigation with half-strength Hoagland culture solution. Net photosynthetic rate, transpiration and stomatal conductance were measured at weekly intervals. The leaf pH was measured to determine whether there was a decrease in pH (leaf acidification) of the leaves during the night, when the plants experienced water deficit stress. All the accessions exhibited marked reduction in all the measured photosynthetic characteristics when experience water deficit stress. However, a measurable CO_2 uptake was carried out by leaves of all the accessions, in the wake of marked decreases in stomatal conductance. There is evidence to suggest that when exposed to water stress *J. curcas* accessions switch from C_3 mode of photosynthesis to CAM photosynthetic pathway. This is attested to by the slightly low leaf pH at night. Thamaga accession exhibited an earlier stomatal closure than the other two accessions. This resulted in Thamaga accession displaying a slightly lower dry weight than both Mmadinare and Maun accessions. It could be concluded that *Ja-*

tropha curcas appeared to tolerate water deficit stress due to its ability of switching from C_3 photosynthetic pathway to the CAM photosynthetic pathway, but with a cost to biomass accumulation, as demonstrated by slightly more reduced CO_2 assimilation by Thamaga accession, than the other two accessions.

Keywords

Jatropha curcas, Biodiesel, Crassulacean Acid Metabolism, Net Photosynthetic Rate, Stomatal Conductance

1. Introduction

There has been a gradual increase in fossil fuel consumption as a result of increased use of transportation and industrialization [1] [2]. This increase is in proportion to the rise in atmospheric carbon dioxide. Due to these challenges, reducing the consumption of fossil fuels in order to diminish carbon dioxide emissions has become a crucial countermeasure for global warming or the greenhouse effect. Thus far, the most promising route has been biofuel production [3].

Jatropha curcas is the most promising source of oil that can be used in biofuel production. *Jatropha curcas* seeds produce 35% oil which can be easily converted to biodiesel. It should be noted that biodiesel is carbon neutral as opposed to fossil fuel, which when burnt, releases net CO_2 into the atmosphere.

Photosynthesis is one of the processes that are most affected by water deficit stress due to a reduction in stomatal conductance, as of the initial responses to water deficit [4] [5]. This reduction in stomatal conductance invariably leads to reduction in photosynthetic activity as a result of the limitation of CO_2 uptake (*i.e.*, in CO_2 assimilation) [6].

A high water use efficiency (WUE), is a strategy that plants adopt under water deficit and an important characteristic of plant response to an arid environment [7]. One of the strategies of increasing WUE is the ability of a plant species to switch from C_3 to CAM photosynthetic pathway, which is common in plant inhabiting arid environments [8].

Jatropha curcas is a C_3 plant, but due to its resilience to drought stress [9], there has been speculation that it could alternate between C_3 and CAM. CAM is characterized by the nocturnal uptake of CO_2, followed by the storage of CO_2 as malic acid in the vacuoles of chloroplast-containing cells. However, experimental evidence to back up this claim, for it to switch from C_3 to CAM has not been elucidated [10].

The aim of the study was in two-fold: 1) to assess photosynthetic characteristic responses of various Jatropha *curcas* accessions and 2) to evaluate its ability to switch from carrying out C_3 photosynthetic pathway to CAM pathway, under water deficit stress.

2. Methods and Materials

2.1. Experimental Setup

Three *Jatropha curcas* seeds were collected from the North (Maun [19˚58'S] [23˚25'E]), South (Thamaga [24˚40'S, 25˚32'E]) and Central regions (Mmadinare [21˚56'S, 27˚37'E]) of Botswana. The seeds were germinated in Petri dishes at 25˚C. After emergence, the seedlings were transplanted to pots, filled with potting soil, with each pot containing one plant. The plants were raised in a greenhouse at 27˚C and relative humidity of 65% - 70% (Envirowatch model, South Africa) and irrigated with half-strength Hoagland culture solution. When the plants were 35 days old, they were separated into the control and water deficit stressed sets. Water deficit stress was imposed by withholding irrigation with half-strength Hoagland culture solution [11].

The control, plants were irrigated with half-strength Hoagland culture solution, in alternate days. Water-stressed plants were re-watered after 15 weeks. The plants were rehydrated (*i.e.*, re-irrigated with half-strength Hoagland culture solution) for 5 weeks.

2.2. Gas Exchange Measurements

Photosynthetic rate ($\mu mol \cdot m^{-2} \cdot s^{-1}$), stomatal conductance ($mmol \cdot m^{-2} \cdot s^{-1}$) and the transpiration rate ($mmol\ H_2O \cdot m^{-2} \cdot s^{-1}$) were measured simultaneously with a portable photosynthetic system gas analyzer (LICOR-6400X, Bioscientific Ltd). These measurements were carried out at weekly intervals on fully expanded leaves [12].

2.3. Water Use Efficiency (WUE)

Water use efficiency (WUE) ($\mu mol\ CO_2\ mmol\ H_2O^{-1}$) was calculated using the following formula [13]:

$$\text{WUE} = \frac{\text{Photosynthetic rate}\left(\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}\right)}{\text{transpiration rate}\left(\text{mmol H}_2\text{O}/\text{m}^{-2} \cdot \text{s}^{-1}\right)}$$

2.4. Leaf Acidification Determination

At 49 days of exposure to water deficit stress (when all the accessions had displayed a marked decrease in relative water content), two fully expanded leaves were boiled in 50% ethanol for 15 minutes. Water was added to maintain the initial extraction volume and boiling continued again for 15 minutes. After cooling to room temperature, the pH of the extracts was measured using a pH meter (EUTECH Instruments, pH 700), where the probe was inserted into the extracted sample and measurement taken [8].

2.5. Total Dry Weight

Dry weight (g) was determined after drying plant material (roots and shoot, separately) at 70˚C, until a constant weight was attained [14].

2.6. Experimental Design and Statistical Analysis

The experiment was completely randomized, designed with five replications. ANOVA was used to determine the effects of water deficit stress treatments. All statistical analyses were performed using STATA10.

3. Results

Thamaga accession exhibited an earlier decrease in net photosynthesis after 35 days of exposure to water stress (DOE), while Maun and Mmadinare accessions displayed a decline after 42 and 49 DOE, respectively (Figure 1). At the point of resumption of watering (112 DOE), Maun and Mmadinare accessions exhibited slightly higher values of net photosynthesis than Thamaga accession. Upon re-watering at 112 DOE, Mmadinare accession displayed the quickest recovery of CO_2 uptake, followed by Maun and then Thamaga accessions. It should be noted that Mmadinare accession, also exhibited the highest recovery value of net photosynthesis, followed by Maun and Thamaga displayed the lowest value.

Thamaga accession showed a stomatal conductance reduction after 28 days of exposure to water stress (DOE) while, Maun and Mmadinare accessions exhibited decrease in stomatal conductance after 35 DOE (Figure 2). Maun and Mmadinare accessions exhibited full recovery of stomatal conductance compared to Thamaga accession on completion of rehydration.

When exposed to water stress, Thamaga accession displayed a decrease in transpiration rate after 28 days of exposure to water stress (DOE) while, Maun and Mmadinare accessions displayed a decrease in transpiration rate after 35

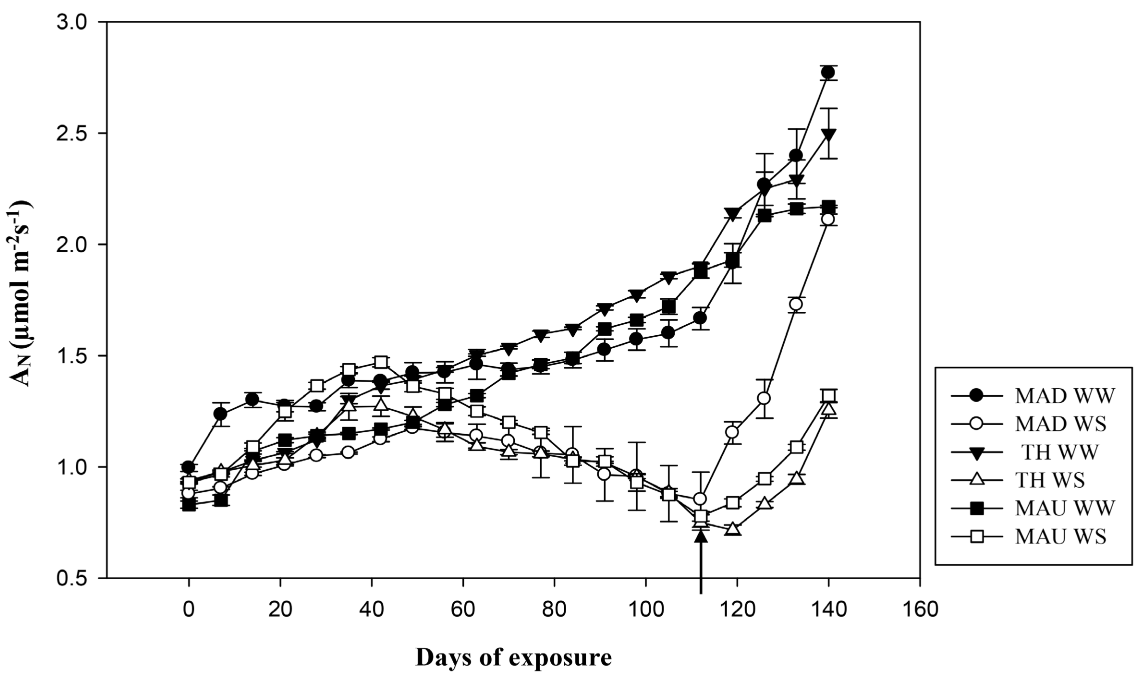

Figure 1. Effect of water stress on Net photosynthesis (A_N) for three *Jatropha curcas* accessions; Mmadinare (MAD), Thamaga (TH) and Maun (MAU). WS denotes water-stressed and WW denotes Control. Bars represent standard error of means (n = 5). Arrow indicates 112 days of exposure (DOE), the beginning of re-watering.

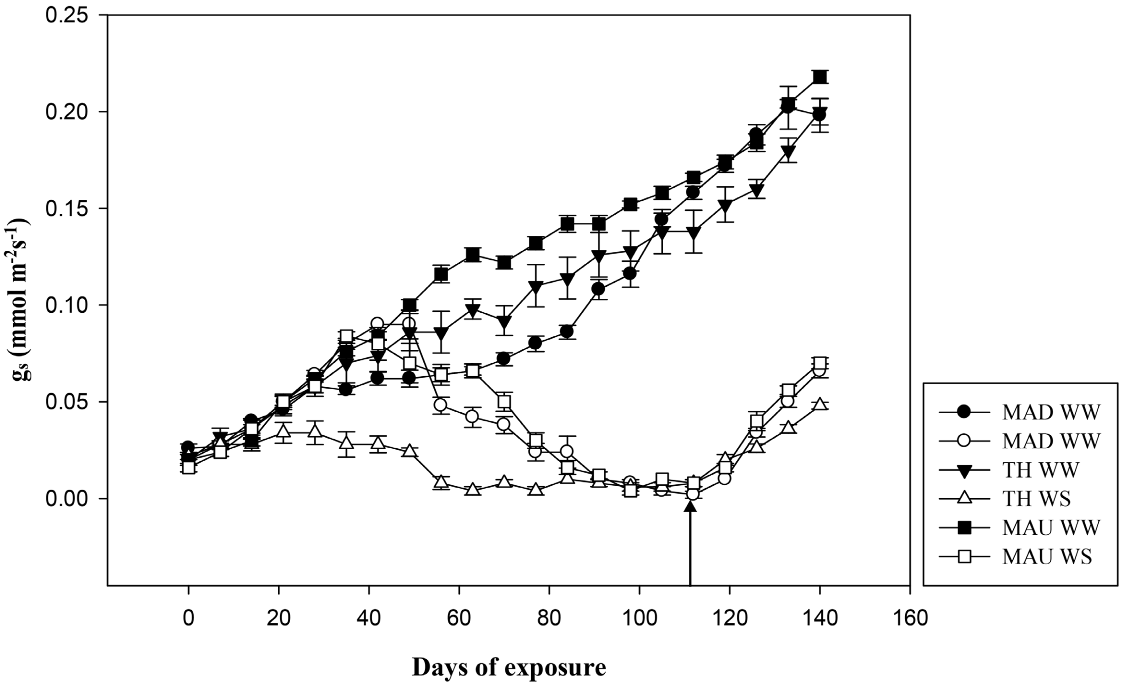

Figure 2. The effect of water stress on the Stomatal conductance (g_s) of three *Jatropha curcas* accessions; Mmadinare (MAD), Thamaga (TH) and Maun (MAU). WS denotes water-stressed and WW denotes Control. Bars represent standard error of means (n = 5). Arrow denotes 112 days of exposure (DOE), the beginning of re-watering.

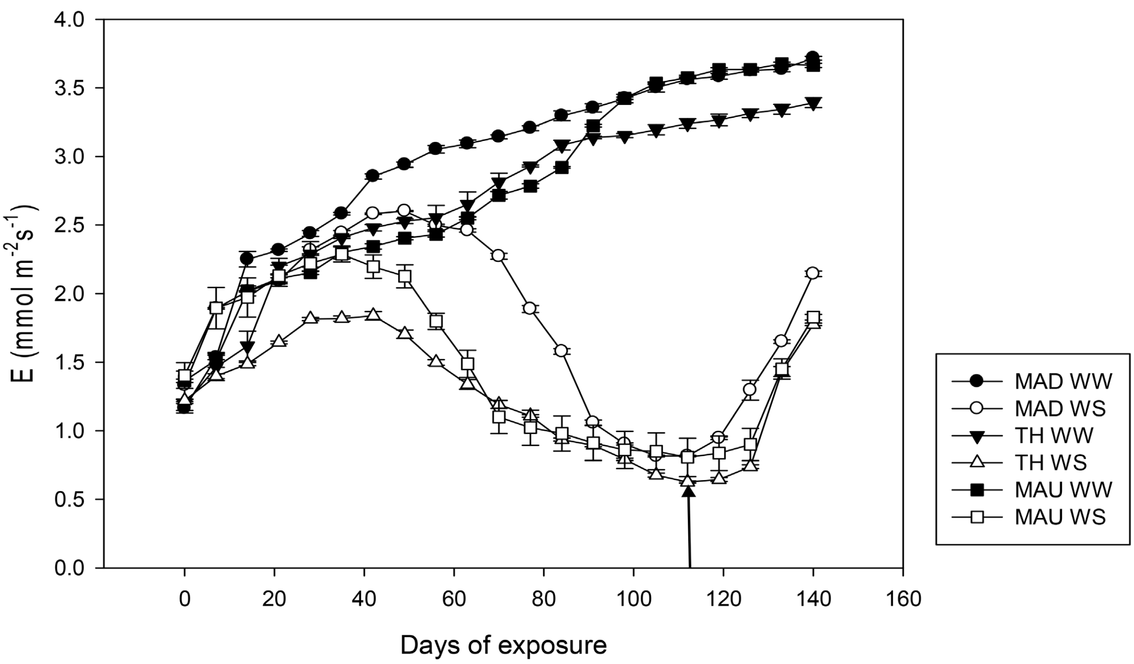

Figure 3. Effect of water deficit on the Transpiration rate (E) of *Jatropha curcas* accessions; Mmadinare (MAD), Thamaga (TH) and Maun (MAU). WS denotes water-stressed and WW denotes Control. Bars represent standard error of means (n = 5), and the arrow denotes the beginning of re-watering at 112 Days of exposure (DOE).

DOE (**Figure 3**). All the three accessions reached their lowest transpiration rate after 112 DOE, with Thamaga accessions, exhibiting the lowest value. Mmadinare and Maun accessions exhibited more rapid recovery in stomatal conductance than Thamaga accession, immediately after resumption of re-watering.

On the 49th day of exposure to water stress, Thamaga accession displayed the lowest pH of 4.94 during nighttime compared to Maun accession and Mmadinare accessions, which displayed pH of 5.64 and 5.34, respectively (Figure 4).

Thamaga accession showed an increase in WUE after 35 days of exposure to water stress (DOE) while Maun accession displayed an increase at 7 DOE (Figure 5).

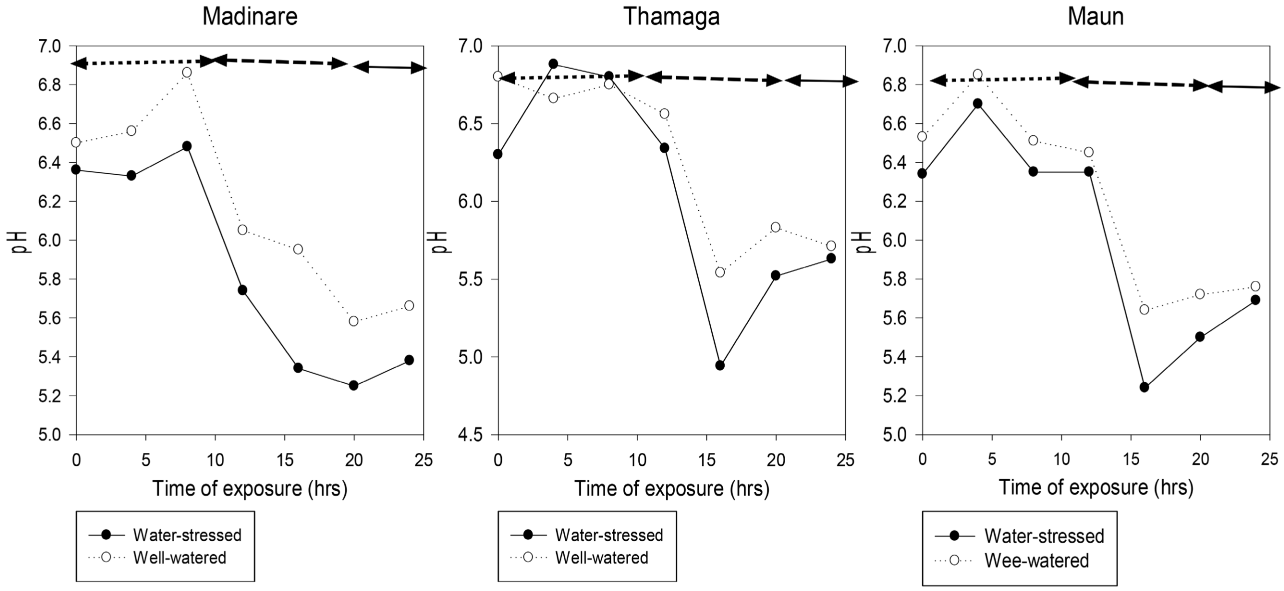

Figure 4. The effect of water deficit stress on the pH of leaves Mmadinare, Thamaga and Maun accessions during daytime (0800 hrs to 1600 hrs), Nighttime (1600 hrs to 1200 midnight) and Morning time (1200 midnight to 0800 hrs). The arrows represent daytime (Dotted), night time (Dashed) and morning time (Straight). Bars represent measures of variability SEM (n = 3).

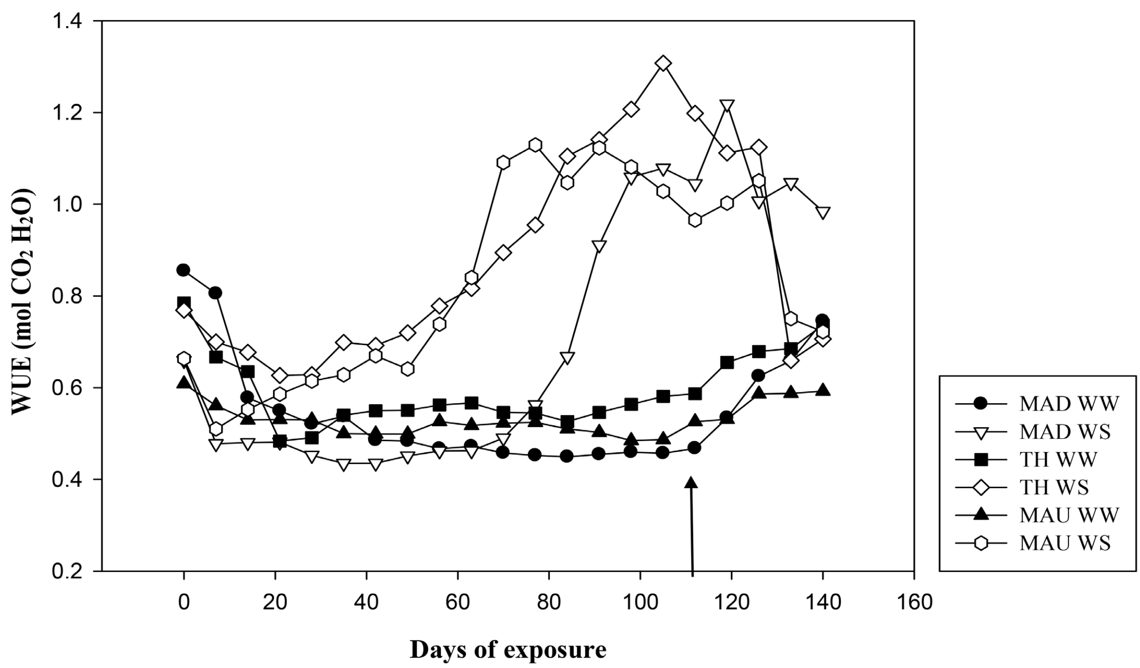

Figure 5. Effect of water deficit stress on the Water use efficiency (A_N/E) in three *Jatropha curcas* accessions *viz.*, Mmadinare (MAD), Thamaga (TH) and Maun (MAU) plants.

Mmadinare accession showed an increase only after 70 DOE. Just before re-watering (at 112 days of exposure to water stress), Thamaga accession showed the highest WUE, followed by Mmadinare accession while Maun exhibited the lowest WUE.

When subjected to water stress, the three accessions exhibited more-or-less the same dry weight (Figure 6). Mmadinare accession displayed a slightly higher mass of 3.00g followed by Maun accession (2.80g) and lastly Thamaga accession (2.74g). There was a marked difference between the control and water stressed plants for each accession.

4. Discussion

The decrease in net photosynthetic rate (Figure 1) exhibited by all accessions, when experienced water deficit stress can be attributed to reduction in stomatal conductance (Figure 2) and decrease in transpiration rate (Figure 3). Interestingly, the reduction in stomatal conductance occurred, without marked decrease in net photosynthesis rate (Figure 1). This suggested that even though the stomata was closed, there was carbon dioxide fixation still taking place, presumably during the night, when the stomata opened. There is evidence to suggest that when exposed to water stress, all the three accession leaves switched from carrying out C_3 mode of photosynthesis to that of Crassulacean Acid Metabolism (CAM), as a strategy for survival. The switch from C_3 photosynthetic pathway to that of Crassulacean acid metabolism (CAM) was demonstrated by the decrease in leaf pH (Figure 4) of the water-stressed plants at night. These results were

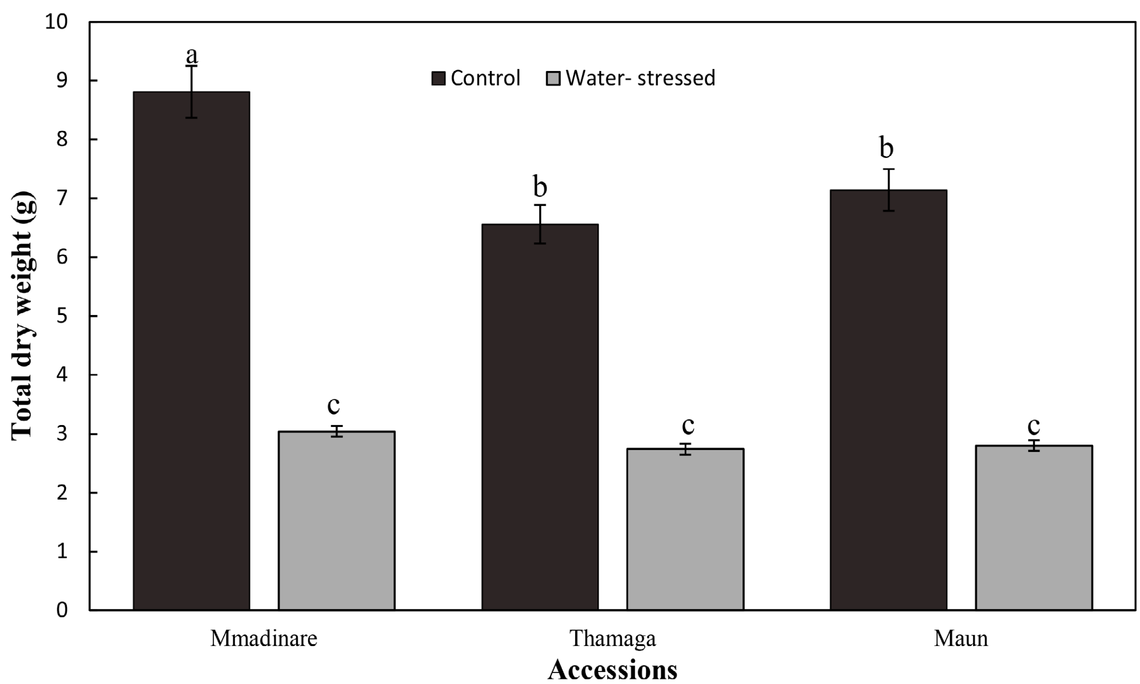

Figure 6. Effect of water stress on the dry weight of the three *Jatropha curcas* accessions (Mmadinare, Thamaga and Maun). Bars represent standard error of means (n = 5). Different letters indicate significant difference (P < 0.05).

consistent with those of Winter and Holtum [8] whereby nocturnal acidification of the *Jatropha curcas* leaf extracts in water-stressed plants was high in comparison to that of the control plants.

Thamaga accession displayed higher WUE (**Figure 5**) than Mmadinare and Maun accessions under water deficit stress conditions. The fact that Thamaga accession was the first to switch from C_3 to CAM photosynthesis pathway, resulted in lowest dry weight (**Figure 6**). CAM species have been reported to have the highest WUE than C_3 and C_4 plants [15]. According to Cushman and Borman [16], rapid and reversible switching between C_3 photosynthesis and CAM can occur within 24 hours in response to environmental changes so drought can induce CAM photosynthesis in facultative species [17]. Similar results have been found in *C. minor,* where CAM was considered useful in the water conservation of the plant during the dry season [18].

5. Conclusion

It could be concluded that *Jatropha curcas* appeared to tolerate water stress due to its increased water use efficiency which appeared to be as a result of switching from C_3 photosynthetic pathway to the CAM photosynthetic pathway in water deficit-stressed plants. The C_3-CAM switch appeared to be more pronounced in Thamaga accession than in Mmadinare and Maun accessions which consequently resulted in Thamaga accession's slightly lower total dry weight.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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