

Effects of Water Deficit Stress on the Chlorophyll Fluorescence of *Jatropha Curcas* Accession

Research Article

Volume 3 Issue 2- 2022

Author Details

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Article History

Received: November 13, 2022 Accepted: November 16, 2022 Published: November 17, 2022

Abstract

The ability of *Jatropha curcas* to grow in marginal and dry soils has not been extensively explored. Given the current world energy problems of high fossil fuel consumption which plays a significant role in the greenhouse effect, *J. curcas* biodiesel have been considered a potential alternative source of clean energy. Although it best grows under precipitation amounts of 250-1200mm annually, this plant has been reported to be drought tolerant, but no study has been done to ascertain this in Botswana. In this study, potted plant (35 days old) from three *J. curcas* accessions namely Mmadinare (Central region), Thamaga (Southern region) and Maun (Northern region) in Botswana, were selected to represent the different the climatic zones with respect to rainfall per annum, were assessed for water stress tolerance. These were subjected to continuous well-watered conditions (control) and to water deficit stress (with-holding water) until they reached wilting point, (followed by a 28-day re-watering period). The maximum photochemical efficiency of PSII (estimated from dark-adapted Fv/Fm ratio) and the maximum quantum yield of PSII electron (Φ PSII) transport were measured at weekly intervals. The most rapid recovery of dark-adapted Fv/Fm ratio and Φ PSII was displayed by Mmadinare and Maun accessions than Thamaga accession. The rapid recovery in Fv/Fm ratio and Φ PSII could be attributed mainly to dissipation of excess energy harmlessly as heat. On the other hand, the slower recovery rate in dark-adapted Fv/Fm ratio and Φ PSII displayed by Thamaga accession showed that the decline could be attributed to both downregulation of PSII and possibly damage to the PSII reaction centres.

Keywords: *Jatropha curcas*; Chlorophyll fluorescence; Dark-adapted Fv/Fm ratio; Quantum yield of PSII electron transport

Introduction

Plant growth has been traditionally measured by destruction methods that include sampling, drying and chemical analysis of dried material. However, the direct measurement of chlorophyll fluorescence has been taken as the most conventional method as it is more instantaneous and non-destructive to plants [1]. Even though the harvesting methods are more suitable in long-term assessment of the plant growth, they are inappropriate for the measurement of short-term carbon gain, which contribute to the carbon exchange measurements [2]. The existence of any type of stress that results in the damage of PSII (photoinhibition), or the induction of sustained quenching [3] results in a lowering of the maximum photochemical efficiency of PSII (estimated by dark-adapted Fv/Fm ratio). Fm is the maximal possible value for fluorescence and the difference between Fo and Fm is the variable fluorescence, Fv [4]. Thus, measuring dark-adapted Fv/Fm following

an appropriate period of dark adaptation has been used as one of the most common techniques for measuring 'stress' in leaves [5].

Dark-adapted Fv/Fm ratio provides a rapid method for determining changes in maximum quantum efficiency of PSII photochemistry [5]. It estimates the efficiency of excitation energy capture by open PSII reaction centres [5]. For unstressed leaves, the value of Fv/Fm is highly consistent, with values of ~0.83, and correlates to the maximum quantum yield of (Φ PSII) [4]. When exposed to water deficit stress, photosynthetic productivity and canopy development significantly drop in most plant species. This could be attributed to the reduction of CO₂ uptake that is associated with increased dissipation of excitation energy in the PSII antennae and photo damage of PSII reaction centers [6].

Jatropha curcas is a fundamental crop in biofuel production because of its seeds that contain about 40% of oil. The *Jatropha curcas*



plant's oil is the most promising substitute to petrol diesel that can be used in biofuel production due to the availability, sustainability and lower feedstock price in the market [7]. *Jatropha curcas* is a perennial plant belonging to the family Euphorbiaceae, thought to originate from Mexico or Central America and was introduced to Africa and Asia in the 16th century [8]. It is a drought-resistant plant that can be cultivated in areas of low rainfall [9]. It is, therefore, essential to study the diurnal measurements of chlorophyll fluorescence of *Jatropha curcas* to assess the extent to which the photosynthetic apparatus is damaged under water deficit stress and see if it can recover.

Materials and methods

Experimental setup

Three *Jatropha curcas* accession seeds were collected from the North, South and Central regions of Botswana (Maun [19°58'S, 23°25'E]; Thamaga [24°40'S, 25°32'E] and Mmadinare [21°56'S, 27°37'E], respectively). They were then germinated in Petri dishes at 25°C. After emergence, the seedlings were transferred to pots filled with potting soil. Each pot contained one plant and each accession had five replicates. The plants were raised in a greenhouse at 27°C and relative humidity of 65-70% (Envirowatch model, South Africa). When the plants were 35 days old, they were separated into the control (watered with half-strength Hoagland's solution) and water deficit stress sets. Water deficit stress was imposed by withholding water. The control plants were watered with 250ml of half-strength Hoagland's solution (composed as shown in Table 1) [10] in alternate days [5].

Table 1: Initial Recovery rates of dark F_v/F_m ratio and Φ PSII in different water-stressed *Jatropha curcas* plants after 7 days of rehydration

Accession	Initial Recovery rate	
	Dark F_v/F_m ratio	Φ PSII
Mmadinare	0.007	0.02
Thamaga	0.001	0.02
Maun	0.003	0.02

Chlorophyll fluorescence

Chlorophyll fluorescence was measured according to Moseki and Dintwe [5]. The efficiency of excitation energy capture by open PSII reaction centres was estimated from the ratio of variable to maximal chlorophyll fluorescence (i.e., dark-adapted F_v/F_m ratio). Fully expanded leaves of four different plants from each treatment were dark-adapted for 15 minutes by placing the plants in a dark chamber and the dark-adapted F_v/F_m was then determined using Hansatech fluorometer (FMS2, Hitchin, UK). Diurnal measurements of dark-adapted F_v/F_m ratio were taken at 0900hrs, 1200hrs and 1500hrs on day 49 of water deficit stress exposure. The maximum quantum yield of PSII electron (PSII) transport was determined using the Hansatech fluorometer (FMS2, Hitchin, UK).

Results

When subjected to water deficit stress, Maun accession exhibited decline in dark-adapted F_v/F_m ratio after 21 Days of exposure to water deficit stress (DOE) (Figure 1). Thamaga accession showed a decline in the dark-adapted F_v/F_m ratio after 35 DOE while Mmadinare accession displayed a decline after 49 DOE (Figure 1). At the end of water deficit stress treatment (112 DOE), Thamaga accession displayed the lowest dark-adapted F_v/F_m ratio compared to the other accessions (Maun and Mmadinare). Mmadinare accession followed by Maun accession showed the most rapid recovery of dark adapted F_v/F_m ratio than Thamaga accession after a week of re-watering at 112 DOE. After

exposure to water deficit stress for 49 days, all the accessions appeared to recover from 1200hrs to 1600hrs in the dark-adapted F_v/F_m ratio (Figure 2). In the morning (at 0900hrs), Maun accession followed by Mmadinare accession exhibited the highest dark-adapted F_v/F_m ratio, with Thamaga accession exhibiting the least dark-adapted F_v/F_m ratio. At noon (at 1200hrs), Mmadinare and Maun accessions still displayed a higher dark-adapted F_v/F_m ratio than Thamaga accession. By late afternoon (at 1600hrs), all the accessions displayed a recovery in dark-adapted F_v/F_m ratio to almost their initial morning dark-adapted F_v/F_m ratio.

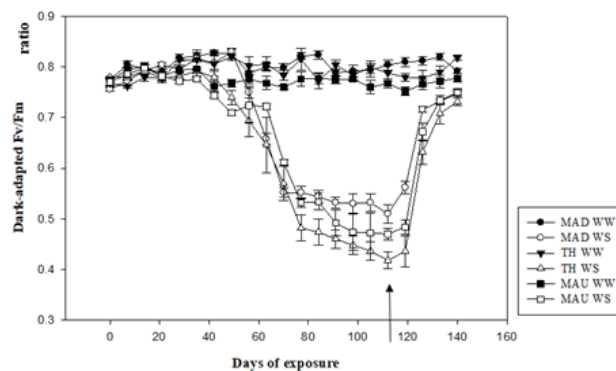


Figure 1: The effect of water deficit stress on the maximum photochemical efficiency of PSII (estimated from dark-adapted F_v/F_m ratio) for Thamaga (TH), Mmadinare (MAD) and Maun (MAU) *Jatropha curcas* accessions. Bars represent standard error of means (n=5). The arrow at 112 Days after exposure denotes the beginning of re-watering.

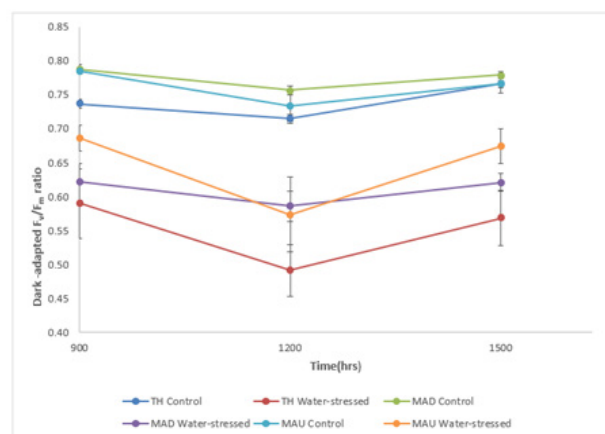


Figure 2: The effect of water deficit stress on the maximum photochemical efficiency of PSII (estimated from dark-adapted F_v/F_m ratio) for Thamaga (TH), Mmadinare (MAD) and Maun (MAU) *Jatropha curcas* accessions measured from 0900hrs, 1200hrs and 1600hrs after 49 days of exposure. Bars represent SEM (n=5).

Water-stressed Mmadinare and Maun accessions exhibited decline in Φ PSII after 28 days of exposure to water deficit stress (DOE) while Thamaga accession displayed a decline after 56 DOE (Figure 3). At the end of water deficit stress exposure (at 112 DOE), Thamaga accession displayed the lowest Φ PSII compared to other accessions (Maun and Mmadinare). Mmadinare and Maun accessions accession showed the most rapid recovery of Φ PSII than Thamaga accession a week after the day of re-watering. All the accessions displayed recovery of Φ PSII to more or less their initial value at the start of the experiment (Day 0). After re-watering, Mmadinare accession displayed a higher recovery rate of 0.007 in dark F_v/F_m ratio than Thamaga and Maun accessions which exhibited recovery rate of 0.003 and 0.001 respectively (Table 1). All the accessions exhibited an equal recovery rate in the quantum yield of PSII electron transport (Φ PSII) of 0.02.



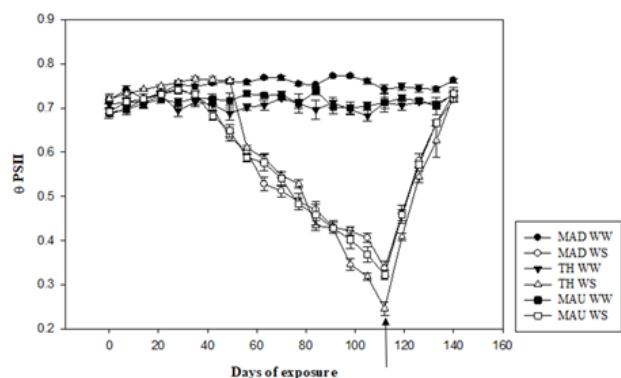


Figure 3: The effect of water deficit stress on the maximum quantum efficiency of photosystem (Φ PSII) in three *Jatropha curcas* accessions viz., Mmadinare (MAD), Thamaga (TH) and Maun (MAU). Bars indicate standard error of means ($n=5$). Arrow denotes the beginning of re-watering (112 DOE).

Discussion

At the end of water deficit stress exposure, all the three accessions exhibited a decrease in dark-adapted Fv/Fm ratio, but it was more pronounced in Thamaga accession than in Mmadinare and Maun accessions (Figure 1). Similar results were obtained by Luvaha et al. [11], who observed an apparent reduction in the dark-adapted Fv/Fm ratio with increasing soil moisture deficit. It is worth-noting that upon re-watering, Thamaga accession displayed a slightly lower recovery rate of dark-adapted Fv/Fm ratio than the other two accessions (Maun and Mmadinare) (Figure 1). The quick recovery of Mmadinare and Maun accession in dark-adapted Fv/Fm ratio could suggest that the decline in the dark-adapted Fv/Fm ratio could be ascribed mainly to the downregulation of PSII or heat dissipation by the Xanthophyll cycle rather than to damage to the PSII reaction centres [12,13]. Diurnal measurements on the 49th day showed that indeed the decrease in dark-adapted Fv/Fm ratio could be ascribed to the downregulation of PSII as attested to by their quick recovery from 12000hrs to 16000hrs (Figure 2). On the other hand, Thamaga accession displayed the lowest initial recovery rate of dark-adapted Fv/Fm ratio after re-watering (Table 1) that could be attributed to both downregulation of PSII and damage to the PSII reaction centres.

The quantum yield of PSII electron transport (Φ PSII), which represents electron flow beyond PSII [5], decreased in all the accessions when subjected to water deficit stress (Figure 3). Thamaga accession displayed a more decreased quantum yield of PSII electron transport than Mmadinare and Maun accessions. These results are consistent with those obtained by Moseki and Dintwe [5] where water-stressed 'Town' sorghum cultivar exhibited a significantly more reduced quantum yield of PSII electron transport than 'Segaolane' sorghum cultivar. All the accessions, after re-watering showed recovery of Φ PSII to almost their initial value (at the start of the water deficit stress exposure, day 0) (Figure 3). Furthermore, all the three accessions exhibited an equal recovery rate in Φ PSII (Table 1). This most rapid recovery of Φ PSII of all the three accessions upon re-watering might indicate that the decrease of Φ PSII could be attributed mainly to excitation energy being dissipated as heat (down-regulation of PSII) by the xanthophyll cycle [14]. These findings were consistent with those of Subrahmanyam et al. [15] who found out that water deficit stress had no significant effect on primary photochemistry of PSII in wheat cultivars.

Conclusion

It could be concluded that the most rapid recovery of dark-adapted

Fv/Fm ratio and Φ PSII displayed by Mmadinare and Maun accessions than Thamaga accession could be attributed mainly to dissipation of excess energy harmlessly as heat. On the other hand, the slower recovery rate in dark-adapted Fv/Fm ratio and Φ PSII displayed by Thamaga accession showed that the decline could be attributed to both the downregulation of PSII and possibly damage to the PSII reaction centres.

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