



Water Stress Limits Growth and Physiological Performance of *Jatropha curcas* L. Seedlings

**Bassirou Sine^{1*}, Bassiaka Ouattara^{1,2}, Diariétou Sambakhé¹,
Alassane Waly Ngom¹ and Aïda Ndiaye¹**

¹*Centre d'Etude Régional pour l'Amélioration de l'Adaptation à la Sécheresse, Institut Sénégalais de Recherches Agricoles (CERAAS/ISRA), Thiès, Sénégal.*

²*Université de Fada N'Gourma (U-FDG), Fada N'Gourma, Burkina Faso.*

Authors' contributions

This work was carried out in collaboration among all authors. Authors BS, BO and AWN designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors DS and AN performed the statistical analysis. Authors BS and BO managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Jatropha (*Jatropha curcas* L.) is a potential biodiesel plant that gained much attention in non-oil production countries. We investigated the ability of the species to cope with water deficit occurring in semi-arid zones like Senegal climatic conditions. The layout of the experiment was a randomized complete blocks design with two factors (level and frequency of irrigation) and 6 repetitions. The trial was conducted from December 2012 to June 2013 in CERAAS/ISRA at Thiès, Senegal. Potted *Jatropha* seedlings were exposed, after three months under similar well-watered conditions, to three water regimes (100%, 50% and 25% of field capacity, respectively C100, C50 and C25) and two frequency of watering once and twice watering a week (F1 and F2, respectively). The different treatments didn't affect significantly collar diameter and plant height during the time of the experimentation. Nevertheless, the trend line is up for collar diameter and plant height in well-watered plants. For the same frequency of irrigation, biomass accumulated was high in C100. No difference was found between plants biomass in C25-F1 and C25-F2. For physiological parameters, there are no differences between the treatments at the onset of stress. However, after

*Corresponding author: E-mail: bassirou.sine@gmail.com;

2 weeks of stress, photosynthesis, stomatal conductance and transpiration were affected permanently in treatments C100-F1, C50-F1 and C25-F1 and their values never reached those of plants of C100-F2, 50-F2 and C25-F2. The opposite was noted for leaf temperature. For the same frequency of irrigation, photosynthesis, stomatal conductance and transpiration were higher in C100 and the opposite occurred for the leaf temperature. These results demonstrate that growth and productivity of *Jatropha* are significantly affected by water stress conditions. Its need to be watered regularly to maintain physiological functions and biomass production definitely highlights that its growth in semi-arid zones is economically unlikely.

Keywords: Biomass; leaf temperature; photosynthesis; transpiration; stomatal conductance.

1. INTRODUCTION

Jatropha (*Jatropha curcas* L.), also called physic nut, is a large shrub or small tree, belonging to the genus Euphorbiaceae, producing seeds that contain oil. The species has its natural distribution in the Northeastern part of South America [1]. It's claimed that *Jatropha* doesn't require arable land as it is able to grow in infertile, moderately sodic and saline soils [2]. There are also claims that it is drought-resistant, thus able to grow in arid and semi-arid areas where it tolerates high temperatures and low soil moisture [3]. Because of these characteristics it has been used in land reclamation and soil erosion prevention [4]. Moreover, global attention on biofuels and the potential for *Jatropha* to produce biodiesel from marginal land with low inputs has recently created an overestimated interest in this species. This has resulted in the planting of large areas of *Jatropha* in Asia, Africa and America. Countries such as India have initiated large-scale plantings of *Jatropha* in efforts towards the increased use of bio-diesel as an alternative to fossil fuel imports [5]. In Sahelian area, *Jatropha* has gained a hype attention and is promoted for its large use. Traditionally, it is used in the protection hedges around arable land and housing. Because of its toxicity, it is not browsed by animals. Its oil is not edible and is traditionally used for manufacturing soap and medicinal applications. Today, its oil is suitable for industrial processing or as an energy source [1].

A major constraint for the extended use of *Jatropha* in water-scarce regions seems to be the lack of knowledge on its agronomic performance specially under water stress conditions. Although *Jatropha* grows in semi-arid and arid tropical areas and can therefore be considered as a drought tolerant species, reliable studies on this species facing water stress are necessary to make responsible decisions on investments.

Progress in plant responses to water stress is more and more important as most climate-change scenarios suggest an increase in water stress in many areas of the world [6]. Drought in conjunction with coincident high temperature and radiation as in Sahelian area, poses the most important environmental constraints to plant survival and crop productivity [7].

A focus on water relation of *Jatropha* seedlings is an important step in understanding the ability of the species to cope with Senegal water stress as this stage is most stressful. The small sized, shallow roots and minimal capacity for resource storage make seedlings less tolerant to the unfavorable environment [8]. Most of the studies that explain the distribution and regeneration of species are based on water relation of seedlings. A better understanding of the effects of water stress on *Jatropha* is useful for improved management practices and breeding efforts in agriculture. This study came about in response to the proposed introduction of *Jatropha* into crop system in Senegal for poverty alleviation, job creation and the provision of alternative energy sources. The present study aims at investigating growth and physiological performance of *Jatropha* in Sahelian climate conditions.

2. MATERIALS AND METHODS

2.1 Plant Material

Jatropha seeds were harvested 2 months early in an old *Jatropha* plantation of the Regional Centre for the Improvement of Adaptation to Drought (Centre d'Étude Régional pour l'Amélioration de l'Adaptation à la Sécheresse; CERAAS) were used.

2.2 Methodology

Seeds were sown in 16 L plastic pot (25 cm height and 25 cm diameter). The bottom of each pot was holed to allow water to drain. Prior to fill pots with soil, we did a fine layer of gravel at the

bottom to avoid holes to be stuck. Pots were filled with a sandy clay soil and their weight determined. Afterward, they were irrigated until field capacity and allowed to drain 24 hours and weighted. For each one, the difference in weight between wet and dry pot was considered as the quantity of water at field capacity. Every day, pots were weighted and irrigated with a quantity of water equivalent to the difference in its weight of the day compared to its weight at capacity. Thus, pots were maintained at field capacity. Three seeds were sown in each pot and two weeks after sowing, only one germinated plant was maintained in each pot.

Collar diameter and plant height were measured in three times during the experiment: end of weeks 1, 8 and 22.

At 3 months old, a set of 36 plants were submitted to water stress and a same number maintained in well-watering. The layout of the experiment was a randomized complete blocks design with two factors (level and frequency of irrigation) and 6 repetitions. Two plants composed a unit. Three levels of irrigation were observed: irrigation at field capacity (C100), irrigation at 50% of field capacity (C50) and irrigation at 25% of field capacity (C25). For irrigation, two frequencies were observed: once a week (F1) and twice a week (F2).

Five weeks before to end experimentation, leaf temperature, photosynthesis, transpiration and stomatal conductance were measured using *CI-340 photosynthesis system of CID Bio-Science*.

At the end of the experimentation, plants were cut at the collar and dry out door for one week. To determine dry biomass, stems and leaves were oven dry at 60°C during 72 hours and weighted on electronic scale.

2.3 Statistical Analysis

Statistical analyses were performed with R software package, version 4.0.2. Data of biomass, collar diameter and plant height were submitted to a two ways ANOVA. Multiple comparisons were done using Tukey test at 5% level. Outcome variables of *CI-340 photosynthesis system* (physiological and weather variables) were tested for linear trends by analysis of covariance (ANCOVA) to determine the relationship between variables and the effects of the level and frequency of watering. When ANCOVA has shown a significant effect,

multiple comparisons were analyzed by Fisher's LSD test with the Bonferroni correction, which was employed for adjusting the significance level to control type I error rates [9]. The TukeyHSD test was performed to determine the value of the LSD.

3. RESULTS

3.1 Growth Parameters

The different treatments didn't affect significantly collar diameter and plant height during the period of the experimentation. Nevertheless, the trend line is up for collar diameter and plant height in well-watered plants (plants watered at 100% of field capacity twice a week) as illustrated in Fig. 1. The maximum collar diameter (24 cm) was noted in F2C100 - plants irrigated at field capacity twice a week and the minimum (21 cm) was recorded in C25 - plants irrigated with 25% of the quantity of water used to reach field capacity.

3.2 Biomass Accumulation

The levels as well as the frequency of irrigation had significant effects on plant aboveground dry biomass at the end of experimentation. For the same frequency of irrigation, biomass accumulated was higher in C100 (Fig. 2). No difference was found between plants biomass in C25 irrigated one or two fold a week. For plants irrigated twice a week, biomass in C100 was 7 g higher than biomass in C50, which was 6 g higher than biomass in C25.

3.3 Physiological Parameters

Water stress had a significant effect on *Jatropha* physiology. Photosynthesis as well as transpiration and stomatal conductance were significantly affected by the level and the frequency of watering. These parameters decline when the quantity of water brought per week to the plant decreased. Independently to the frequency of watering, plants in well-watered conditions recorded the highest values of photosynthesis, transpiration and stomatal conductance compared to plants maintained watered at 50% field capacity. The lowest values of physiological variables were recorded in plant watered at 25% capacity.

Physiological parameters are higher in plants watered twice per week compared to plant watered one fold per week. Hence, at the end of experimentation (5 weeks of water stress

implementation), photosynthesis in plants C50 irrigated twice a week was $14.69 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ while photosynthesis in plants in C50 irrigated one fold per week was $7.01 \mu\text{mol.m}^{-2}.\text{s}^{-1}$. This one closely follows the plants watered to 100% of its capacity. Similar trends were observed in stomatal conductance and transpiration (Fig. 3). In comparison of all the treatments, the highest value was recorded in plants irrigated at field capacity twice a week. Irrigated plants once a week showed a higher leaf temperature than those irrigated twice a week.

When water is brought to C100-F1 the level of photosynthesis as well as transpiration and conductance don't reach those of plants irrigated at field capacity twice a week. Thus, physiological parameters are affected permanently in plants irrigated at 50% at field capacity one fold per week and their values never reached those of plants with similar level of watered twice per week. The measured physiological parameters didn't show most of the time a significant difference in C25 irrigated one or two fold per week.

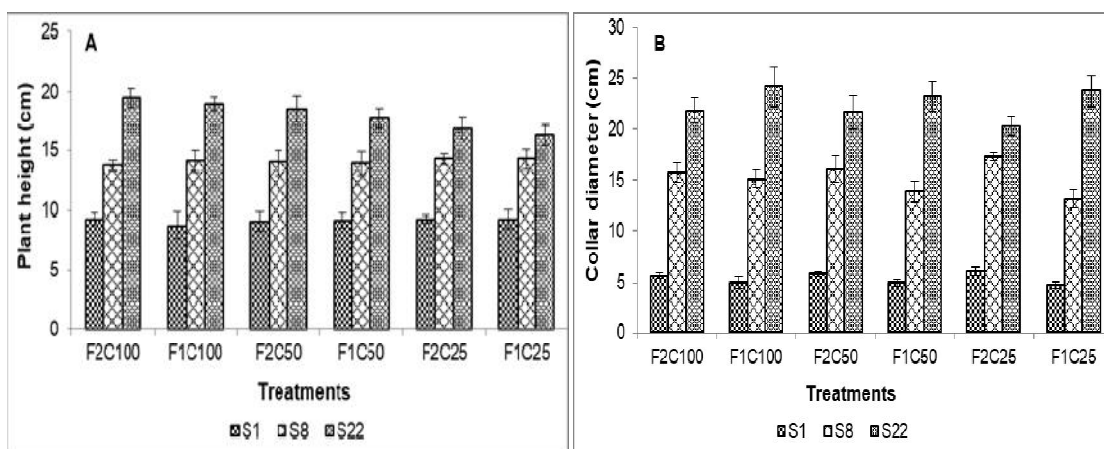


Fig. 1. Evolution of plant height (A) and collar diameter (B) during the experimentation S1, S2 and S3 = 1st, 8th and 22nd week of experimentation, respectively. C100, C50 and C25 = plants watered at 100%, 50% and 25% of field capacity, respectively. F2 and F1 = plants watered twice and once a week, respectively. I = standard error.

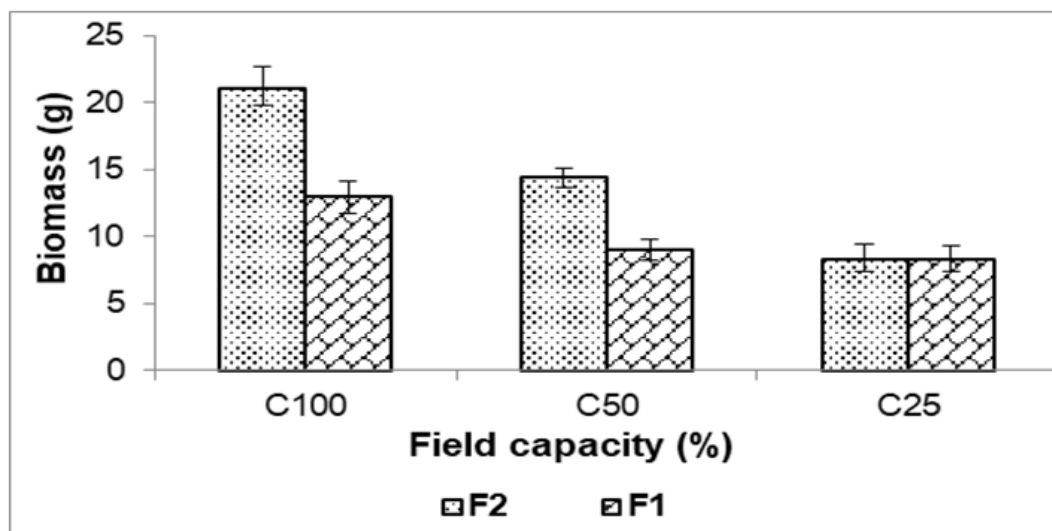


Fig. 2. Aboveground dry biomass C100, C50 and C25 = plants watered at 100%, 50% and 25% of field capacity, respectively. F2 and F1 = plants watered twice and once a week, respectively. I = standard error.

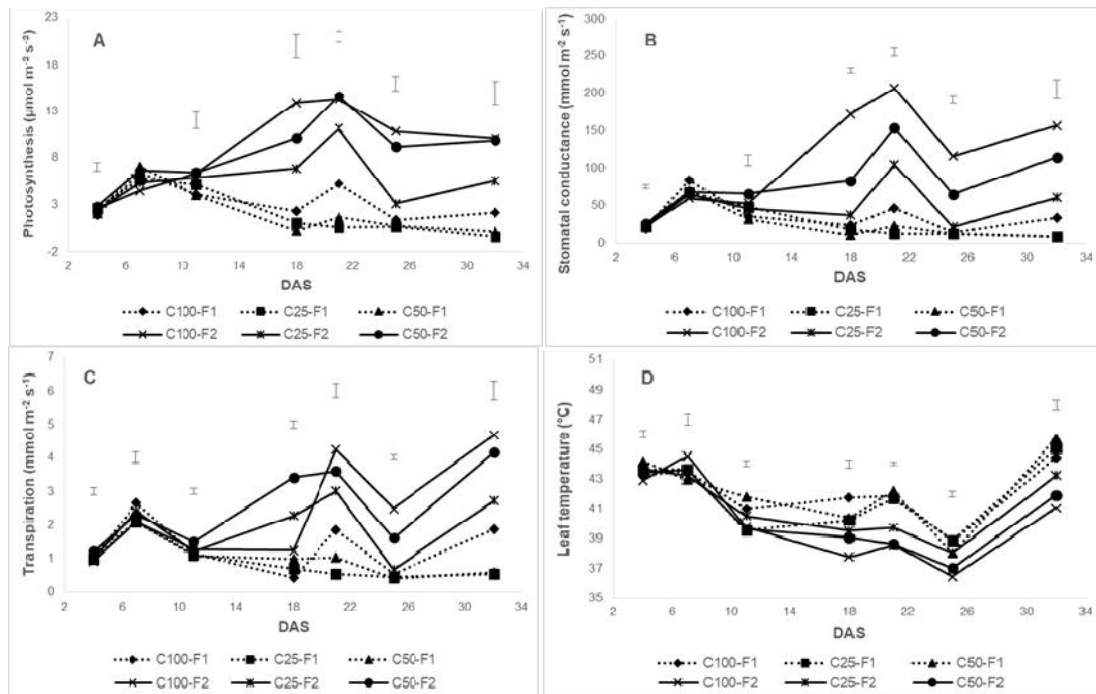


Fig. 3. Evolution of photosynthesis (A), stomatal conductance (B), transpiration (C) and leaf temperature (D) during stress

C100, C50 and C25 = plants watered at 100%, 50% and 25% of field capacity, respectively. F2 and F1 = plants watered twice and once a week, respectively. DAS = day after stress. I = LSD (least significant difference).

4. DISCUSSION

An adequate supply of water is as essential to the successful growth of plants as photosynthesis [8]. In this study, seedling growth is better in pots watered twice a week at 100% field capacity. In the treatments C50 and C25, aboveground dry biomass and physiological parameters (photosynthesis, conductance and transpiration) dropped. The leaf temperature negatively correlated to the other physiological parameters is increased. This suppose that growth of *Jatropha* in Sahelian area (suggested to water stress) without watering, its agronomical performance could be affected significantly, especially when humidity is lower than 50% field capacity. As it was shown in this experiment, on at plant watered at 25% of field capacity, the biomass and physiological parameters, except leaf temperature, are drastically reduced. Leaf temperature negatively correlated to transpiration, obviously increased. Under glasshouse, it is reported that drought significantly reduced leaf area, biomass and relative growth rate of *Jatropha* seedlings [10]. In

South Africa, a reduced increase in *Jatropha* height when rainfall decreases is noted [11]. According to authors, plants can avoid water stress by maximizing water uptake or minimizing water loss [12].

In the present study, stressed plants strategy was to minimize water loss by reducing plant transpiration. Indeed, one of the earliest responses of plants to water shortage is to close stomata in order to prevent excessive loss of water [13]. But, a negative consequence of stomatal closure is to limit CO₂ diffusion rate and thus photosynthesis. This is the critical dilemma "lose water to fix carbon" that plants face [14]. By closing stomata, conductance and photosynthesis are reduced and biomass production too [15]. This confirms the fact that plants respond to water stress through modification of suite physiological characteristics [16,17].

Jatropha seedlings watered at 100% and 50% field capacity twice a week showed better growth compared to those in the same water regimes

watered once a week. It has been reported with potted seedlings that shoot growth is directly related to watering frequency, and the growth of plant is usually reduced under the condition of water stress [18]. Because the supply of nutrients to a plant is directly related to water movement in roots, and when such movement ceases because of lower soil moisture availability, roots are limited to those nutrient ions within the range of diffusion, this supply must become limiting within a very short time [18,19]. Similar results have also been reported on *Prunus davidiana* under water stress [20]. The authors showed that water stress affected dry matter accumulation. In addition, the watering regimes significantly affected all these growth properties. Significant differences between water regimes in net photosynthesis, transpiration and stomatal conductance have also been recorded. Working with two species of *Prosopis* (*Prosopis argentina* and *Prosopis alata*) under water stress, a decrease in total biomass, biomass of leaves, stems and roots, leaf area, and number of leaves under water stress conditions was observed [21].

The behavior of leaf conductance followed very closely that of photosynthesis as shown by the close correlation between both parameters. In potted olive trees, stomatal conductance has shown limited photosynthesis in trees subjected to mild and moderate water stress [22]. Soil water status plays an important role in controlling leaf conductance in *Jatropha* seedlings as in olive trees [23,24].

When water is available, we recorded high transpiration. Therefore, when water is available, large numbers of *Jatropha* trees, in hedges or plantations, could result in an increase in water use as canopy transpiration will be increased considerably. Indeed, a large canopy (high leaf area index) increases the transpiration rate considerably. But in the case of low moisture, *Jatropha* growth is negatively affected. So, the growing of *Jatropha* in large scale could result in an environment problem (rapid decline of soil water) or a problem of growth and productivity of the species in water stress environment.

5. CONCLUSION

Water stress is a major factor limiting growth and development in higher plants. As water stress is a common feature in many environments, many plant species have developed various mechanisms to cope with restricted water supply. Our results show that *Jatropha* has avoidance strategy to cope with water stress. The results

are so unequivocal that *Jatropha* under the experimental conditions does not fulfil the claims that it is a wonder biodiesel plant, able to grow and produce anywhere. However, it can survive in scarce-water regions without being a profitable investment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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