

Effect of the soil water content on *Jatropha* seedlings in a tropical climate

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Abstract. The purpose of this study was to evaluate growth, chlorophyll content, and photosynthesis in *Jatropha* at different levels of soil moisture. Plants were cultivated in containers and the treatments of the soil water content evaluated were: 0% (without watering), 20, 40, 60, and 80% soil water content. Plant height was statistically similar for all treatments, but the number of leaves differed significantly. Total dry matter and chlorophyll at 40, 60, and 80% soil water content were statistically similar, but different from 0 and 20% soil water content. Leaf area at 40, 60, and 80% soil water content was statistically different from 0 and 20% soil water content. The photosynthetic rate, transpiration and stomatal conductance at 60 and 80% soil water content were statistically similar but different from 0 and 20% soil water content. Water stress affected growth, chlorophyll content, photosynthetic rate, transpiration, and stomatal conductance.

Key words: *Jatropha*, water stress, biodiesel, photosynthesis, Mexico

INTRODUCTION

Jatropha (*Jatropha curcas* L.) is a perennial, deciduous, stem-succulent shrub which produces seeds rich in oil that can be converted into biodiesel (Brittaine and Litaladio, 2010). This species is easy to establish as it has a fast growth, requires minimal amounts of water and survives in poor soils (Henning, 2004; Valdés-Rodríguez *et al.*, 2011). However, there is a misconception that *Jatropha* is adapted to grow well under a low water regime and under drought stress. To achieve a good yield, *Jatropha* needs certain good management practices, such as plant nutrition, minimizing loss of water through evaporation, and cutting (Behera *et al.*,

2010; Jongschaap *et al.*, 2007). The enhancement of biomass production under drought stress is a key factor for *Jatropha* when it is cultivated on available marginal soils.

From a sustainability perspective, *Jatropha* as a source of biofuels provides opportunities for extra income, empowerment of women, sustainable rural energy production, and protection and restoration of soils. Small-scale mixed cropping of *Jatropha* with food- or cash-crops may provide added benefits of *Jatropha* avoiding a negative environmental impact (Asselbergs *et al.*, 2006).

Optimal amounts of water at the right time are crucial for optimal plant growth and production (Sánchez-Díaz and Aguirreola, 2000a). The effect of water stress on growth and yield is well known in most commercial crops. Indeed, drought is generally recognized as a principal limiting factor controlling plant growth (Maes *et al.*, 2009). For example, plant photosynthesis, transpiration, chlorophyll and dry matter production are closely related processes, influenced by water deficits (Sarker *et al.*, 2005). But very little information has been generated related to this issue in *Jatropha*. Indeed, no quantitative data on water demand, water productivity, and water use efficiency in *Jatropha* are available at present (Achten *et al.*, 2008). Most studies do not reveal the effect of changes in the plant-water relations on growth, chlorophyll, and photosynthesis, although its roots have high demands for aeration (Ouwens *et al.*, 2007) and the plant is sensitive to soil pathogens under high soil moisture (Valdés-Rodríguez *et al.*, 2011).

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Jatropha can survive using minimal water compared to other crops. Abou and Atta (2009) reported water use efficiency for seed and oil yields increasing positively with water application and an average water consumption rate of $6 \text{ dcm}^3 \text{ week}^{-1}$ throughout the growing season. On the contrary, Said-Al Ahl *et al.* (2009) found that oregano oil production was affected by the amount of available water and nitrogen fertilization, concluding that high fresh matter and essential oils were obtained with 80% soil moisture. Not so for other species, as Khalid (2006) concludes that excess of soil water (125%) and 50% favoured an increase in the percentage of essential oils, total carbohydrates, while protein levels were affected.

Hence, this study was designed to evaluate the response of Jatropha growth, the chlorophyll content, and the photosynthetic rate at different levels of the soil water content (SWC) during the vegetative phase.

MATERIALS AND METHODS

The outdoor experiment was conducted from October to December 2009 in Veracruz, Mexico ($19^\circ 16' 00'' \text{ N}$ and $96^\circ 16' 32'' \text{ W}$; 18 m a.s.l.). This took place inside a plastic greenhouse to handle unforeseen rains that might affect or alter soil moisture. The seeds used were collected in Veracruz ($18^\circ 59' 52'' \text{ N}$ and $96^\circ 15' 31'' \text{ W}$, 27 m a.s.l.) during 2009.

On the 15th of October, Jatropha seeds were sown in a sand substrate. After germination, seedlings with well-developed roots and having the same height (4.5 cm) were selected and one single plant was transplanted into a black plastic container filled with 6 kg mixture of soil, sand, and compost (2:1:1). Once the seedlings were transplanted (October 24), the plants were watered for 15 days before being subjected to the treatments.

The treatments evaluated were different soil moisture levels. A total of five treatments were assessed: 0 (without added water), and 20, 40, 60, and 80% SWC. The experimental design was completely randomized with seven replications for a total of 35 plot units. Water was provided using a graduated cylinder, applying just the missing water related to each treatment. From this moment onwards, a strict control of the soil water content was verified gravimetrically and with the help of a tensiometer (Aquate Instrument

E300), which directly provided the soil water content in % from the soil. The gravimetric water content corresponding to the assigned treatment correlated closely with tensiometer readings. In fact, the tensiometer was calibrated using the gravimetric moisture content method. Soil samples of approximately 20 g were dried placed in an oven heated to 105°C for 24 h, and after drying, the containers were again weighed and the mass of water determined as before and after the readings. Knowing the bulk density, it was possible to determine soil water contents. Therefore, the water required for each treatment was supplied with a cylinder up to being inside the corresponding range ($\pm 5\%$ SWC).

The plants were cultivated in conditions of a 12:12 h light/dark regime at an average temperature of 35°C and a quantum flux of $537.99 \text{ W m}^{-2} \text{ s}^{-1}$ (Table 1).

The plants did not receive nutrient solutions or fertilizers.

Plant height and stem diameter (at 6 cm height from the soil) were measured every week over two months using a ruler of 30 cm and a digital caliper (accuracy 0.01 mm). The number of leaves was counted and leaf area was estimated using the model obtained by Liv *et al.* (2007).

The chlorophyll content was measured on young, completely expanded leaves with a SPAD Minolta model 502, from 12:00 h to 14:00 h. Net photosynthesis (A), transpiration (E) and stomatal conductance (g_s) was determined with a BioScientific model LCpro+ portable on clear and sunny days from 12:00 to 14:30 h.

At the end of the experiment, the roots were thoroughly washed and the plants were separated into shoots (leaves, stems) and roots and dried to constant mass in an oven-dried at 80°C . The shoot: root ratio was calculated for each treatment.

One-way analyses of variance (ANOVA) using STATISTICA 7.1 was applied (StatSoft., 1984-2006). A Tukey-test was used to compare means ($p=0.05$) and confidence intervals (0.95) bars were calculated.

RESULTS AND DISCUSSION

The ANOVA for plant height indicates that there was no statistical difference among the treatments for all samplings (Fig. 1). ANOVA results for the stem diameter showed

Table 1. Temperature (maximum and minimum), relative humidity, and solar radiation during November and December, 2009

Month	Date	Temperature ($^\circ\text{C}$) (max-min)	Relative humidity (%)	Solar radiation ($\text{W m}^{-2} \text{ s}^{-1}$)
November	1-15	30.6-15.3	76.2	538.8
	16-30	30.3-15.7	79.5	538.1
December	1-15	32.9-15.4	81.7	539.8
	16-31	30.4-12.6	82.0	535.2

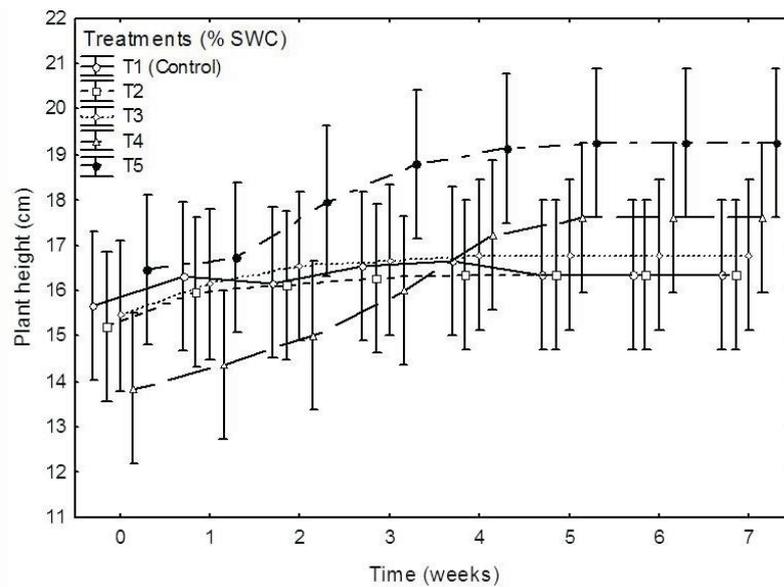


Fig. 1. Effect of SWC on plant height in *Jatropha* during seven weeks. Vertical bars denote 0.95 confidence intervals.

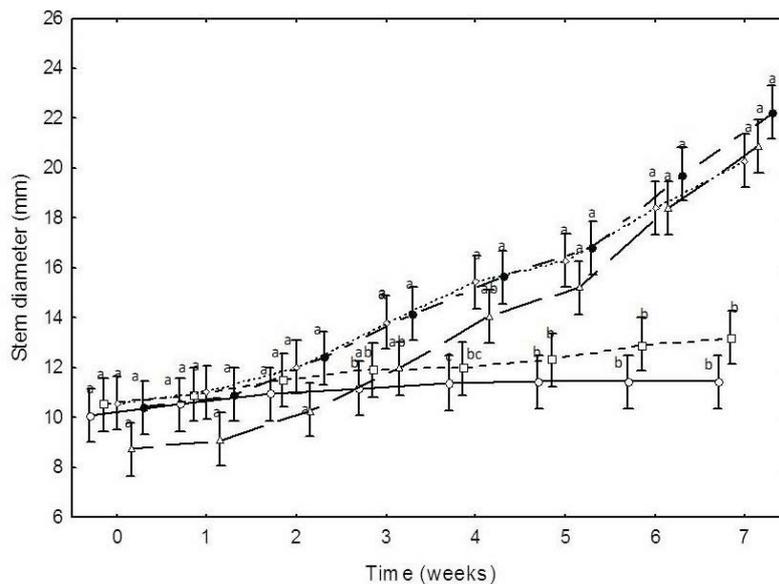


Fig. 2. Response of stem diameter of *Jatropha* at different levels of SWC during seven weeks. Means with the same letter are not significantly different ($p \leq 0.05$). Explanation as in Fig. 1.

significant differences ($p < 0.05$) among the treatments from the third (21 days) to the seventh sampling (49 days). At 80% SWC, from the sixth sampling (42 days) onward, the trend was different in comparison to the other treatments, achieving 22.23 mm in diameter. The treatments at 40 and 60% SWC were statistically similar to the sixth sample onward, achieving 18.40 and 18.41 mm, respectively. The same effect was found for T0 (without added water) and 20% SWC (Fig. 2).

Significant differences ($p < 0.05$) between the treatments were obtained for the number of leaves after five weeks. The highest value was found at 80% SWC, with eight leaves

after seven weeks. The remaining moisture levels had different results, with the least being T0 (without added water) and that for 20% SWC, as the plants tended to drop almost all their leaves (Fig. 3). This indicates that in response to water stress and to avoid evapotranspiration, *Jatropha* drops its leaves (Devkota and Jha, 2011; Sánchez-Díaz and Aguirreola, 2000b). It should be noted that although the plants dropped their leaves they did not die, as normally happens to other plants. This is an indication of the drought tolerance that this plant species possesses (Fujimaki and Kikuchi, 2010), and is evidenced by the succulent stems remaining green.

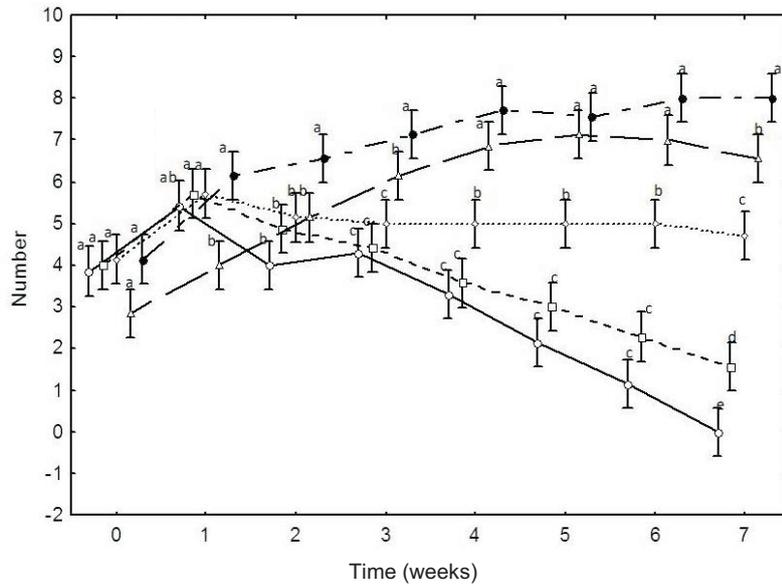


Fig. 3. Effect of the SWC on the number of leaves in *Jatropha* during seven weeks. Means with the same letter are not significantly different ($p < 0.05$). Explanation as in Fig. 1.

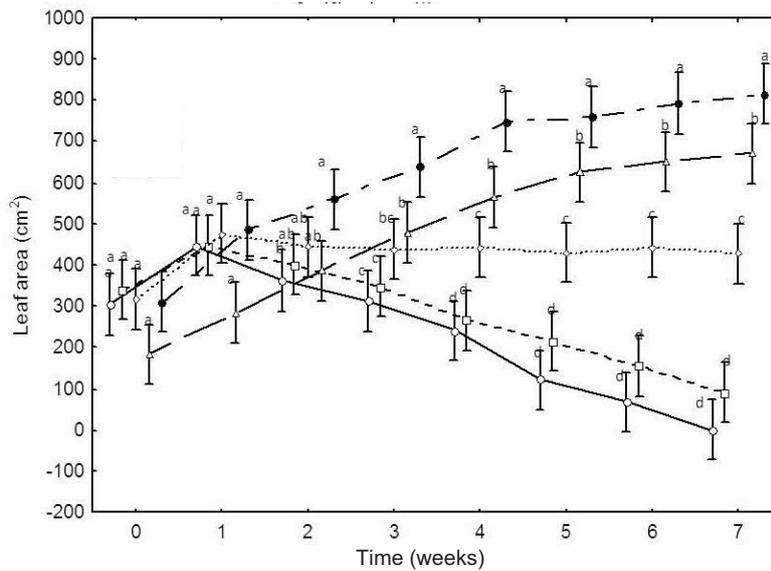


Fig. 4. Effect of the SWC on leaf area in *Jatropha* at different levels during seven weeks. Means with the same letter are not significantly different ($p < 0.05$). Explanation as in Fig. 1.

The ANOVA indicated highly significant differences (Tukey-test, $p < 0.05$) among the treatments from the third week onward for leaf area. The availability of water in the soil strongly influenced this variable. At 80% SWC, more leaf area was produced. Treatment T0 (without added water) had the lowest value, initiating defoliation during the second week of treatment application (Fig. 4). In this regard, Devkota and Jha (2011), working with *Centella asiatica* found higher leaf area with 125% of pot water capacity, but under water stress the leaf area was lessened, as it happened to 30% of pot water capacity. Mean comparisons indicated

that T0 and 20% SWC were statistically similar in all samplings. On the other hand, treatments of 40, 60, and 80% SWC had different effects on leaf area from the second week onward. In this matter, Monclus *et al.* (2006) mentioned that a first response to water deficit in the plants is reducing the leaf area and growth, thereby losing less water.

All dry matter results were calculated based on the last sampling. For all the treatments, the stems had the driest matter, and the values increased positively with soil moisture. Above- and below-ground biomass increased positively with soil moisture, with the greatest values at 80%

Table 2. Effect of different levels of the SWC on dry matter (g) of stem, leaves, root, shoot, shoot: root ratio and total dry matter in *Jatropha*

Treatments (% WC)	Dry matter of				Shoot: root ratio	Total dry matter
	stem	leaves	root	shoot		
0 (Without adding water)	8.47c ± 3.78	0.00d ± 0.00	0.93c ± 0.33	8.47b ± 3.78	8.74a ± 1.94	9.40b ± 4.08
20	7.41d ± 1.57	0.56d ± 0.30	1.27c ± 0.24	7.97b ± 1.61	6.39c ± 1.55	9.24b ± 1.73
40	12.16c ± 3.99	4.60b ± 0.55	2.50b ± 1.07	16.76a ± 4.08	7.60c ± 3.1	19.26a ± 4.56
60	18.54a ± 3.53	2.80c ± 0.30	2.89b ± 0.48	21.34a ± 3.58	7.54c ± 1.72	24.23a ± 3.59
80	13.76b ± 2.12	5.88a ± 0.24	4.07a ± 0.54	19.64a ± 1.89	4.89b ± 0.91	23.71a ± 1.94

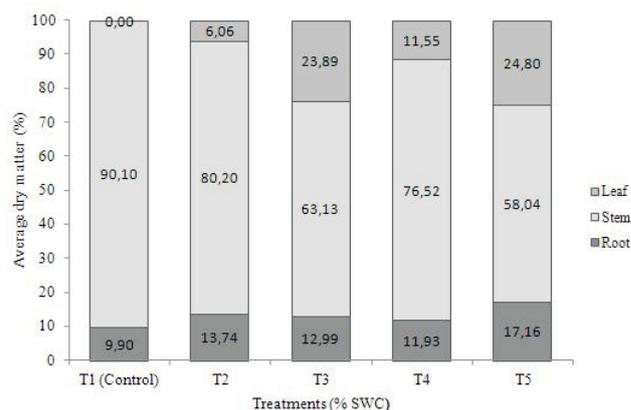
Means with different letters in the same column are statistically different ($p \leq 0.05$).

SWC (Laribi *et al.*, 2009). These results are alike with Khalid (2006) results. The shoot: root ratio at the end of the experiment showed that treatments with 20, 40, and 60% SWC had similar trends, had a greater effect than T0 (without adding water), and had the lowest value at 80% SWC. The T0 treatment had the highest root: shoot ratio (Table 2).

The percentage of dry matter distribution was completely different in comparison to that expressed in grams. At low levels of the soil water content, values for stems increased and were greatest in T1 with 90.1% dry matter of stem. In leaves and roots, the high SWC levels (80%) produced the highest dry matter. All the treatments were similar for root production, with 80% SWC having the highest value (Fig. 5). Stems are used for water storage by seedlings in this species, allowing them to survive dry periods (Brittaine and Lutaladio, 2010). Thus, the larger ratio in T1 could be an indication of water stress, which also depressed stem and leaf growth.

The ANOVA showed significant differences in the chlorophyll content among the treatments from the third week onward (Fig. 6), there was no significant difference between 40, 60, and 80% SWC in the last week, resulting in a mean value of approximately 38.00 SPAD units. It is possible that soil moisture among 40 to 80% SWC did not affect nutrient assimilation and maintained a greater concentration of chlorophyll. However, treatments T0 (without added water) and 20% SWC were affected, because leaf senescence, wilt, and yellowing reflect nutrient deficiencies due to water stress and therefore a low chlorophyll content (Devkota and Jha, 2011).

Table 3 presents the mean rates of photosynthesis and their statistical comparisons according to Tukey-test ($p \leq 0.05$). In the absence of added water (T0) and at 20% of SWC, the responses were statistically similar ($p > 0.05$), as were the re-

**Fig. 5.** Effect of SWC on dry matter production in *Jatropha*: T1 – control, T2 – 20, T3 – 40, T4 – 60, T5 – 80% SWC.

sults for 60 and 80% SWC. This is expected because for CO_2 to penetrate the leaves the stomata must be opened, a process regulated by water stored by the cells. Therefore, given sufficient water in the soil, stomatal opening will be reduced and photosynthesis increased, in contrast to the treatments without adding water and that for 20% SWC (Miyashita *et al.*, 2005).

The analysis of variance showed significant differences among the treatments for transpiration. Mean comparisons (Tukey-test, $p \leq 0.05$) revealed that transpiration decreased with increased water stress, and there was a statistically similar increase in transpiration at 60 and 80% SWC (Table 3). This result was expected because the soil water content is positively correlated with water absorption, favouring stomatal opening and more transpiration. Stomatal closure reduces transpiration and the amount of water consumed by the plant

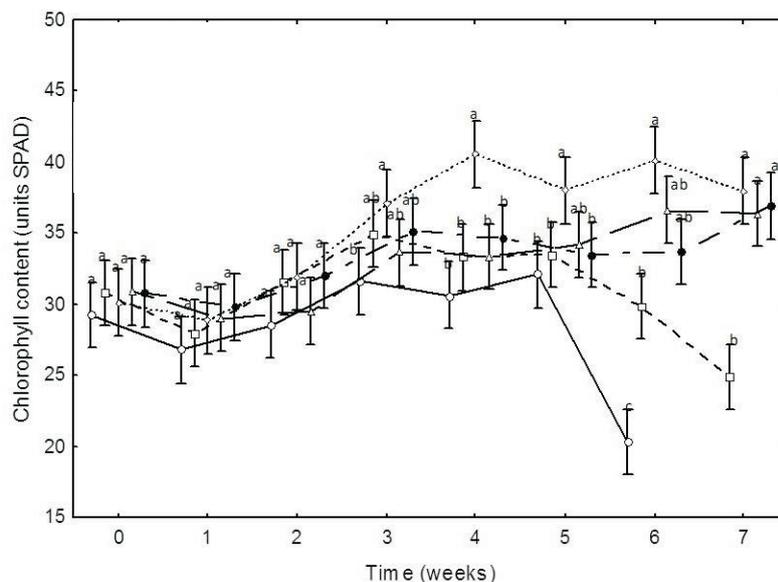


Fig. 6. Effect of the soil water content on the chlorophyll content in *Jatropha* during seven weeks. Means with the same letter are not significantly different ($p \leq 0.05$). Explanation as in Fig. 1.

Table 3. Photosynthetic rate, transpiration, and stomatal conductance in *Jatropha* at different levels of SWC (%)

Treatments (% WC)	Photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Transpiration ($\text{mmol m}^{-2} \text{s}^{-1}$)	Stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$)
0 (Without adding water)	-0.004c	-0.001c	0.00b
20	0.50c	0.13c	0.004b
40	7.63b	2.68b	0.10a
60	11.88a	5.18a	0.21a
80	11.52a	4.96a	0.20a

Means with the same letter are not significantly different ($p \leq 0.05$).

(Tambussi, 2004). It is well known that photosynthesis and transpiration are regulated by a stomatal feedback control mechanism which is influenced by water deficits (Cornic, 2000; Pompelli *et al.*, 2010).

Stomatal conductance is closely related to soil moisture, since in the treatments (40, 60 and 80% SWC) where there was more available moisture the stomatal conductance was higher (0.10, 0.11 and 0.20 $\text{mmol m}^{-2} \text{s}^{-1}$, respectively), unlike in the treatments without water (T0) and 20% of available moisture (Table 3). This means that when plants are subjected to water stress, they partially or temporarily close their stomata and consequently reduce their growth and yield (Said-Al Ahl *et al.*, 2009).

CONCLUSIONS

1. The growth of *Jatropha* was similar and favoured between 60 and 80% SWC, with bigger stems, number of leaves, leaf area and total dry matter. In the treatments

without adding water and 20% SWC, the grow response was lower and statistically similar for all variables. In plants receiving more than 40% SWC, the chlorophyll content was higher in comparison with plants with 20% or less SWC. Therefore, it is assumed that these levels induced water stress in *Jatropha*. Water stress promoted increased leaf drop, leading to reduced levels of photosynthesis, transpiration, and stomatal conductance. It can be established that the requirement for water in *Jatropha* is moderate and it can withstand long periods of drought by dropping most of its leaves to reduce transpiration. However, to establish commercial plantation of *Jatropha*, plants should be irrigated to promote higher growth rates and yields.

2. It is recommended to explore the soil water content among 60 to 80% to determine the optimum level for *Jatropha*. Further work on water stress at the molecular level also is important to identify tolerance genes, and thus select drought-tolerant varieties for cloning.

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