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Soil texture effects on the development of *Jatropha* seedlings – Mexican variety 'piñón manso'

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ABSTRACT

There is considerable disagreement on the effects of different soil conditions on the growth of *Jatropha curcas* L., although different reports agree on some factors, such as a preference for sandy soils over clay soils. This paper presents results on the growth of seedlings of a low-toxic Mexican cultivar in three different soils from the SE of Mexico. The Mexican seeds were sown in soils of different textures: sandy, sandy-loam and clay-loam. The germinated seedlings were monitored during a period of one month. Then, they were uprooted to record below and above ground morphological parameters and dry matter weight. Stem lengths, root collar diameter, length and diameter of the five main roots, number of true leaves and dry matter were analyzed using one-way ANOVA. The soils used were subjected to a chemical analysis to determine soil nutrient contents, which revealed that clay-loam soil had the highest nutrient content, followed by the sandy-loam, while sandy soil presented the lowest values. It was found that seedlings grown in sandy-loam soil were significantly taller than those in clay-loam and sandy substrates ($P \leq 0.05$). Root collar diameter, root diameter, number of true leaves and dry matter weight did not present significant differences between plants grown in sandy-loam and clay-loam soils, however they showed significant differences when compared with plants grown in sandy soil. Therefore it can be concluded that development of seedlings in sandy-loam soils was greater, followed by those in clay-loam, while the poorest performance of the seedlings was recorded in sandy soil.

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1. Introduction

For more than a decade many governments, organizations and NGOs, including the World Bank; the International Institute of Plant Genetic Research; Technical Aid Organizations from Austria, Netherlands and Germany; and the Rockefeller Foundation, among others, have been promoting the planting and use of certain plants for oil production [1–4]. Among these plants is *Jatropha curcas* L., a *Euphorbiaceae*, and a perennial, stem-semi-succulent shrub or small tree that grows up to 6 m in height and is considered to have its origin in México and Central America [5–7]. This species has gained importance due to the high oil content of its seeds and the fact that this oil can be processed for use as a diesel fuel substitute [8]. Currently, most of *J. curcas* plants distributed in tropical areas around the world are reported to be toxic to humans and domestic animals. The toxicity of the seeds is due to the presence of the toxic protein curcin and to diterpene esters,

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with the latter not degraded by high temperatures [1]. In Mexico there is a non-toxic or, very slightly toxic form of *J. curcas* [1,7,8], which is known as “piñon manso” (mild pinenut). Certain communities from the southeast part of the country eat the roasted seeds of this plant as part of their traditional dishes [9]. Comparative research on toxic and non-toxic seeds has found that those considered non-toxic and eaten by native communities contain a maximum of 110 mg kg^{-1} of diterpene esters [9]. Analysis of the physical and chemical properties of the seed oil, between toxic and non-toxic genotypes, has revealed only minor differences in the proportion of total fatty acids [10]. Due to these characteristics, the non-toxic form of *J. curcas* could represent both a source of biofuel and a food supplement. However, the successful establishment of commercial plantations requires a full understanding of the soil requirements of this species, an aspect which is still being researched. Since the non-toxic form of *J. curcas* is not as widely documented as the toxic form, a search of the published research was conducted on the species alone. The following information was found:

This shrub is deciduous, sheds its leaves during the dry season and it is able to survive long dry periods, therefore it is considered to be well adapted to arid and semi-arid conditions [8,11]. It is also mentioned that although *J. curcas* prefers light and well drained soils, it grows well anywhere, even on gravel, sandy and saline lands. It can grow on the poorest, stony land, even among cracks in stone and on hilly slopes where its seeds are dispersed [1]. Reports from Central America agree that this plant is normally found in marginal soils of low nutrient content [12,13]. Therefore it is commonly recognized that *J. curcas* is normally developed on arid and semi-arid soils [11,14]. Regarding specific soil textures and plant development, the available information suggests that the plant is well adapted to sandy and loamy soils, while clay soils are unsuitable for *J. curcas* if water logging or saturation occur [15]. Other concurring authors indicate that heavy clay soils, like vertisols, are less suitable due their impairment or limitation of root system development and should be avoided when cultivating *J. curcas*. Light sandy and loamy soils appear to be more suitable [2,16]. It is also known that the *J. curcas* root system performs well under arid and semi-arid conditions and has the ability to grow in degraded and marginal soils [17]. Studies on *J. curcas* distribution and development in China mention that *J. curcas* is not sensitive to soil type and it can grow on slightly acidic soil, particularly in lateritic soil, dry red soil, and deep fertile soils with favorable aeration [18].

There is evidence from a study in northwest India that *Jatropha* is tolerant to saline irrigation water, although yield under these conditions has not been fully documented [19]. Growth under conditions of alkaline soils has been widely reported, but soil pH should be within 6.0–8.0 [16]. Additional information reveals that the best soils for *Jatropha* are aerated sand and loam soils deeper than 45 cm in depth [20].

Regarding soil nutrient contents, research conducted on wastelands in India has found that in a density of 2500 plants ha^{-1} an application of 60 kg ha^{-1} of N after two years of growth increased plant height by 23%, plant canopy by 31%, total above ground dry matter by 32% and seed yield by 88% as compared to unfertilized plants; and application of 30 kg ha^{-1} of P_2O_5, for the same period, increased plant height by 17%, plant canopy by 24%, total above ground dry matter by 45% and seed yield by 50% [21]. Other plant productivity studies in India at a planting density of 1666 stems per ha report a consumption of 22 kg of N, 5.0 kg of P, and 8.0 kg of K per ha to produce 1.0 tonne of dry seed [22]. Organic fertilizer, as *Jatropha* de-oiled cake, applied in 1667 plants ha^{-1}, and containing 96 kg of N, 36 kg of P_2O_5 and 42 kg of K_20 per ha, also reported to increase seeds yield up to 93% over control treatment [23]. Lack of fertilization was found to be detrimental for plant production [23]. However, over-application of any kind of fertilization can depresses yields [8].

In Mexico, the official agency of National Environment and Natural Resources (SEMARNAT) [24] states that the edaphological requirements of *J. curcas* range from medium to coarse textures implying a high potential for crop development, indeed even cultivation in saline soils can have some potential. However, sodic and highly saline soils are less suitable. A study of crops sown in the southwest part of the country in 13 plots of land demonstrated that, in the absence of fertilizers, the annual average height increase in sandy soil was 60 cm, in loam 100 cm and in clay 120 cm, indicating reduced growth in sandy soils as compared to loam and clay soils. In addition to this data, tests performed on the same soils regarding levels of NO_3, P and organic matter (OM) found no correlation between these nutrient contents and average plant height. Nevertheless, they found taller plants growing in soils with a pH between 5.8 and 6.4 as compared with those in pH levels higher than 6.5 [25].

From the above findings, it can be surmised that the natural soil requirements for *J. curcas* have not been completely defined. Therefore, there is insufficient information on seedling development, and for the non-toxic form of *J. curcas*, no information was found. Additionally, although *J. curcas* is a native plant in Mexico, little is actually known about it on a scientific level within this country. In fact, *J. curcas* germplasm in the southeast part of Mexico has been collected from different types of soils ranging from sandy to clay and sandy-loam [26], but this data has not been correlated with plants development in the sampled areas. In many respects it remains a wild plant, and in many parts of Mexico it is simply used for live fences and for medicinal purposes [9]. However, if the price of biodiesel rises in the international market, the incentive to cultivate crops such as *J. curcas* or other biodiesel sources may extend to any particular type of soil. For these reasons, the main objective of this study was to determine the natural effects of different tropical soils on the above and below-ground development and growth of Mexican non-toxic *J. curcas* seedlings. The substrates selected were sandy, sandy-loam and clay-loam soils.

2. Materials and methods

2.1. *Experimental site and environmental conditions*

This study was carried out during April 2010 at the Colegio de Postgraduados in the state of Veracruz, Mexico (19°16′00″ N, 96°16′32″ W and 18 masl). In order to avoid rain disturbances, the experiment was conducted inside a greenhouse which was ventilated by two open walls. No artificial conditions were imposed. The maximum, minimum and average

Table 1 – Average daily watering rate for each soil type.

Soil Type/Period of time	Irrigation depth applied in mm			
	Week 1	Week 2	Week 3	Week 4
Sandy	8.09	14.85	17.08	22.87
Sandy-loam	7.72	13.36	20.05	23.39
Clay-loam	7.13	12.62	19.30	21.62

temperatures registered were 40.2 °C, 19.8 °C and 24.9 °C, respectively, while the average relative humidity was 74.78%, EV was 0.478 mm and sun radiation was 688 W m^{-2}.

2.2. *Biological material*

Non-toxic *J. curcas* seeds harvested from the central part of Veracruz state during July 2009 (18° 59′ 52″ N, 96° 15′ 31″ W and 17 masl) were used. The seeds belonged to five, two year old trees located in an orchard. For each plant, 400 g (about 500 seeds) of ripe fruits (yellow), were harvested and the seeds obtained manually. Once the seeds were obtained, they were dried in a shaded and aerated location for 5 h. This process was repeated for three days, until seeds were completely dry. Subsequently, and prior to be used in the experiment, seeds were kept in storage cloth bags in a cool, dry place (18 °C). Determination of non-toxicity was made based on local information that these seeds were used to prepare traditional dishes. Seeds were measured and weighed in order to obtain homogenous seedlings; the selected seeds were those which weighed between 700 mg and 800 mg; resulting in an average seed weight of 742.0 mg (±24.3 mg), length of 18.5 mm (±0.68 mm) and width 10.3 mm (±0.30 mm).

2.3. *Soil characterization and analysis*

Three different soil types were selected for their granulometric characteristics and for being representative of the soils found in the Mexican tropics. Sandy-loam and clay-loam soils were obtained from land belonging to the Colegio de Postgraduados, while sandy soil was obtained from outside the city of Veracruz (19°11′04″ N and 96°14′21″ W). The upper 30 cm of soil was extracted and one sub-sample of 500 g was taken from each soil for analysis. The granulometric characterization was performed following Bouyoucos [27]. The sandy soil had 96% sand, 2.5% silt, and 1.5% clay; the sandy-loam soil had 66% sand, 21% silt, and 13% clay; and the clay-loam soil had 30% sand, 35% silt, and 35% clay [28]. Analysis of pH was conducted using an electronic potentiometer; organic matter was determined by the Walkley–Black method [29] adapted to Mexican soils [30]. The total nitrogen (N) content was determined from organic matter (OM) content (O.M. × 0.05 = N) [31]; Phosphorus (P); and calcium (Ca) and magnesium (Mg) were determined by Ref. [30] methodologies.

2.4. *Experimental design*

A total of three treatments consisting of different soil textures were evaluated: sandy, sandy-loam and clay-loam. The experiment was carried out using a randomized complete block design, with 15 replicates per treatment, providing a total of 45 plot units. One seed per pot was sown into a black polyethylene bag (40 cm × 40 cm size) which was filled with the designated soil type. The germination date was registered in order to accurately record each plant's age. With the aim of correctly measuring above and below-ground parameters from a fast growing plant like *J. curcas* [1,24], a period of 30 days was established based on both our field experiences of root elongation and according to the container size.

2.5. *Irrigation*

The soil in each pot was watered daily up to the field capacity of each soil type to maintain constant soil moisture levels in all containers. For each pot and soil type, the volume of daily watering was registered with the help of a graduated cylinder. Table 1 shows the average daily watering rate during the period.

2.6. *Uprooting and measurement techniques*

Thirty days after germination, the plants were uprooted and the following procedure was performed for each plant:

From previous experience it is known that each substrate adheres to the roots in a different manner. Plants located in sandy and sandy-loam soils were uprooted using water at a slight pressure. Clay soils stick heavily to the roots when soil humidity is either high or low, but when soil humidity is around 50% the substrate can be easily removed. This level of humidity was maintained in the soil immediately prior to the uprooting process and the plants located in clay-loam soils were uprooted without the use of water under pressure.

Stem and root lengths were measured using a measuring tape (accurate to 1 mm) and diameters using a digital caliper (accurate to 0.01 mm). Shoot length was measured as the distance between the root collar and the apical meristem. The five main roots [15] of each plant were measured in length and diameter at the beginning of their base. In addition, the root collar diameter, maximum stem diameter, number of true leaves and number of branches were recorded. Finally, the

Table 2 – Soil chemical analysis performed on each substract.

Soil type	pH	Organic matter g kg^{-1} of dry soil	Total N g kg^{-1} of dry soil	P g kg^{-1} of dry soil	Ca mmol kg^{-1} of dry soil	Mg mmol kg^{-1} of dry soil
Sandy	7.81 (slightly alkaline)	1.68 (very low)	0.084 (very low)	0.126 (low)	77.174	154.348
Sandy-loam	7.26 (Neutral)	39.00 (low)	1.950 (low)	0.046 (medium)	175.395	294.664
Clay-loam	7.43 (slightly alkaline)	72.62 (medium)	3.631 (medium)	0.124 (high)	329.743	519.169

Table 3 – Variables measured in seedlings harvested 30 days after germination. Mean values and standard deviation are given. The statistical test applied in each case is indicated.

Treatment (type of soil)	N	Stem height (mm)	Method: Tukey	Root collar diameter (mm)	Method: Tukey	Number of true leaves	Method: Dunn	Number of branches	Method: Dunn
Sandy	15	124.8 ± 19	C	9.8 ± 1.5	B	2.7 ± 1.4	C	0.2 ± 0.3	B
Sandy-loam	15	226.5 ± 35	A	11.7 ± 1.7	A	7.3 ± 2.5	A	0.8 ± 0.8	B
Clay-loam	15	178.6 ± 23	B	10.8 ± 0.7	AB	6.0 ± 3.3	AB	1.8 ± 0.7	A

Means within a column which do not share the same letter are significantly different ($P < 0.05$).

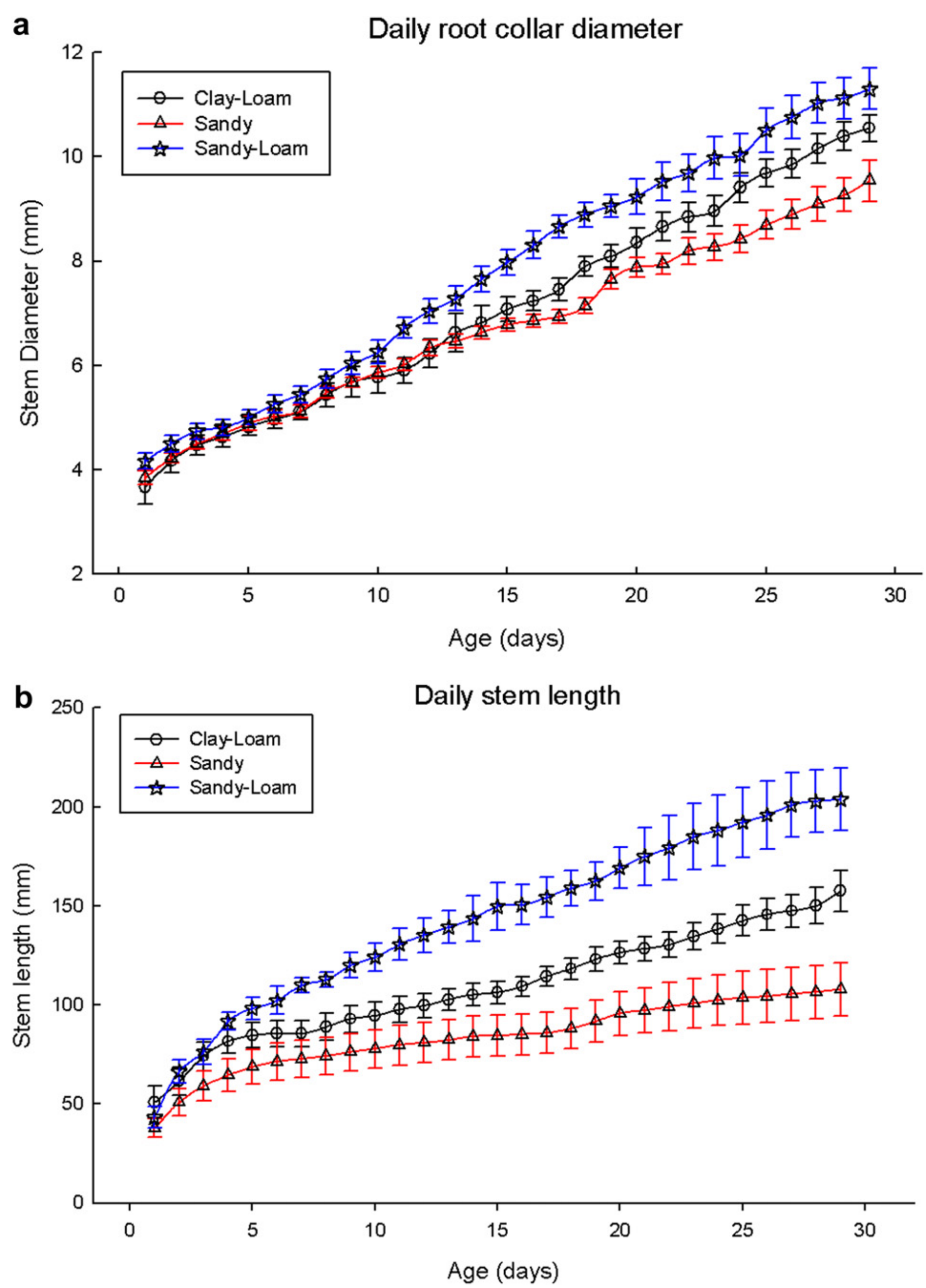

Fig. 1 – Incremental growth of *Jatropha curcas* root collar diameter and stem length when grown in different substrates. a) Root collar diameter. b) Stem length. Differences between plants growing in different substrates began after the sixth day in stem lengths while differences between root collar diameters were observed after 20 days. Error bars represent the standard error of the mean ± S.E.

whole plant was oven-dried at 70 °C for 72 h to determine plant dry mass.

2.7. Statistical analysis

Statistical analysis was performed by one-way analysis of variance with multiple comparison tests ($P < 0.05$) using the program SigmaPlot 10.0. Normality and equal variance tests were conducted and normally distributed data were analyzed with parametric tests, while non-normally distributed data were analyzed by applying the non-parametric Dunn's method, both at $\alpha = 0.05$ level of significance.

3. Results and discussion

3.1. Soil analysis

The results of the chemical analysis of the three soils used are presented in Table 2. All soils were found to be in the alkaline range, which should render them suitable for this study as [16] and [24] have reported the ability of *J. curcas* to grow well in alkaline soils. The clay-loam soil had the highest OM and N contents and also had the highest proportions of Ca and Mg, conversely, the sandy soil exhibited the lowest OM and nutrient contents. Interpretation of these results indicate that clay-loam soil had the highest nutritional contents [32] as N (3.6 g-kg^{-1}) was 1.9 times higher than sandy-loam soil and 43 times higher than sandy soil; Ca (329 mmol kg^{-1} of dry soil) was 1.9 and 4.3 times higher than sandy-loam and sandy soils, respectively; while Mg (519 mmol kg^{-1} of dry soil) was 1.8 and 3.4 times higher than sandy-loam and sandy soils, respectively.

3.2. Effect of substrates on plant above ground parameters

The analysis of variance showed statistical differences (Tukey, $P < 0.05$) in stem height values between the sandy-loam and the other two treatments. Root collar diameters

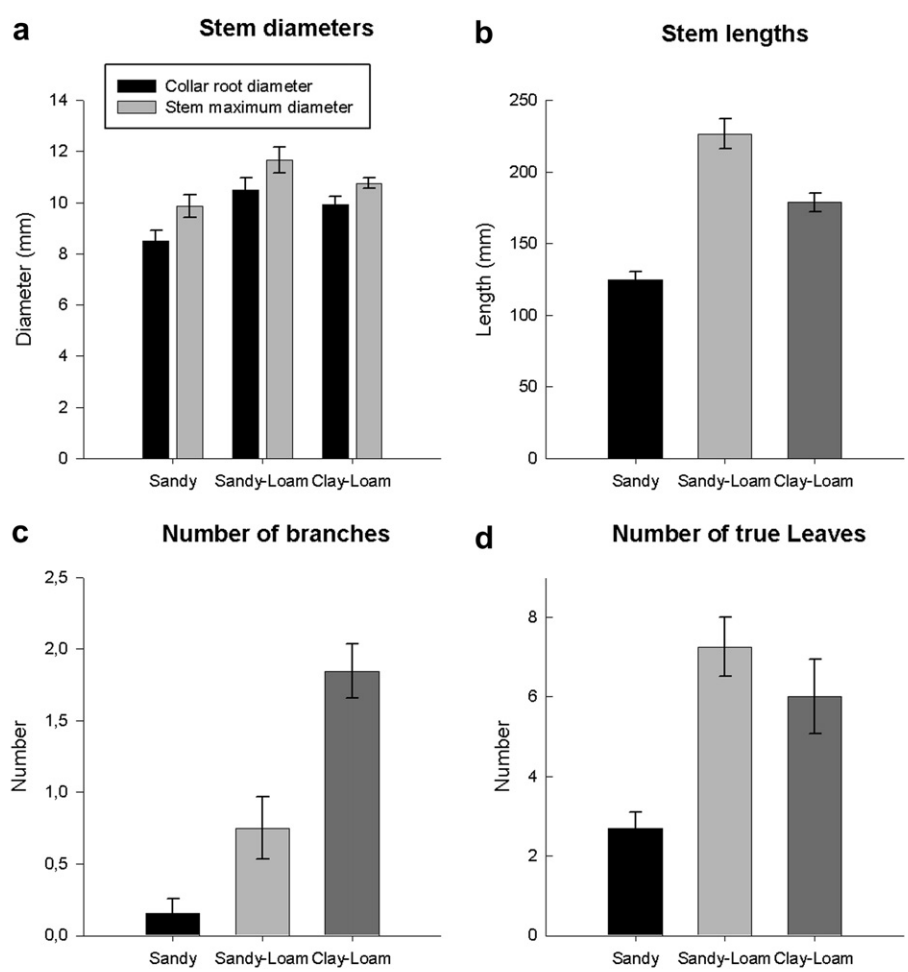

Fig. 2 – Patterns of *Jatropha curcas* characteristics when grown in different substrates: a) Root collar diameters and maximum stem diameters; b) stem lengths; c) Number of branches; and d) Number of true leaves. In general, plants sown in Sandy-loam soil reported the highest performance compared to those located in clay-loam and sandy soils. However, plants located in clay-loam soils reported the highest number of branches compared to the ones located in other soils. Error bars represent one standard error of the mean ± S.E.

Table 4 – Average root lengths and diameters from the five main roots of 30 days old *J. curcas* seedlings.

Treatment (type of soil)	N	Average root length per plant (mm)	Method: Holm-Sidak	Average root diameter per plant (mm)	Method: Holm-Sidak
Sandy	15	316.9 ± 113.5	A	3.712 ± 0.886	B
Sandy-loam	15	322.3 ± 134.4	A	4.548 ± 0.887	A
Clay-loam	15	247.1 ± 73.4	A	4.598 ± 0.245	A

Means within a column which do not share the same letter are significantly different ($P < 0.05$).

and number of true leaves showed statistical differences between all three substrates. Plants grown in the sandy-loam soil produced the greatest stem lengths and diameters and had the highest number of true leaves as compared with the other soils (see Table 3 and Figs. 1 and 2). These results concur with those of Refs. [16,19,24], regarding the preference of the plant for growth on light sandy to loamy soils. However, the poor performance in a sandy soil obtained in this study is contrary to that reported in Refs. [1,8,20]. On the other hand, growth in the clay-loam soil resulted in the highest number of branches in young seedlings, a characteristic which is related to a higher number of inflorescences [5,33]. It is important to note that, although the clay-loam soil used in this experiment had the highest nutrient content, even greater than the sandy-loam soil; other than the number of branches, the best plant performance was obtained in the sandy-loam soil. In this case, it is highly probable that the root systems of these plants were able to more easily obtain nutrients from sandy-loam substrates than from clay-loam substrates, even though the latter had a higher nutrient content. This response may result from the high soil aeration requirements of this species and the fact that clay-loam substrates provide less air capacity than sandy-loam substrates.

3.3. *Effect of substrates on root elongation and thickness*

Although the highest lengths were recorded in sandy-loam soils and the smallest lengths were registered in clay-loam soils (see Table 4), the average root length measured in the five main roots was not statistically different among treatments ($P = 0167$). Significant differences were found in average root diameters (Holm–Sidak; $P = 0.026$), the thickest ones located in clay-loam and sandy-loam soils, and the thinnest ones located in sandy soils (see Table 4). These results show that root development was higher in sandy-loam and clay-loam soils, as compared with sandy soils. An important observation is that *J. curcas*'s root system elongation was similar in the three soils. Similar elongation in sandy and in sandy-loam and clay-loam soils may represent an effort by plants located in sandy soils to reach more soil nutrients, especially N [34], at the expense of significantly thinner roots as has been reported for *Zea mays* roots [35]. Although, root diameter is also affected by the physical properties of the soil [35], and *J. curcas*'s roots concurred with this reference having thicker roots as the soil strength increases.

3.4. *Effect of substrates on dry mass*

The root, stem and leaf dry weights from seedlings growing under sandy-loam substrates were significantly higher ($P \le 0.05$) than those grown in sandy soils. There were no statistical differences between sandy-loam and clay-loam soils (see Table 5 and Fig. 3). Specific reports of similar performance in biomass generation under clay-loam and sandy-loam soils were not found. However, study results showing clay type substrates providing the same performance as sandy-loam substrates, as shown in this experiment, differ from the reports found in Ref. [16]. The poor performance obtained in sandy substrates seem to be contrary to the general perception that *J. curcas* develops better in sandy soils than in clay soils [8,16]. The key point in analyzing this data is to consider that the sandy substrate showed the lowest nutrient content and the plants seem to be very sensitive to this deficiency. It is known that in order to obtain a high biomass, plants normally present high demand for nitrogen and phosphorus [34]. Therefore it is clear that soil quality will certainly affect the growth, yield of seeds and oil production.

Table 5 – Dry matter in *Jatropha curcas* grown in three different soil types. Mean values and standard deviation are given. The statistical test method used in each case is indicated.

Treatment (type of soil)	N	Roots (mg)	Method: Dunn	Stem (mg)	Method: Tukey	Leaves (mg)	Method: Dunn	Total weight (mg)	Method: Dunn
Sandy	15	516 ± 40	B	1352 ± 92	B	927 ± 56	C	2795 ± 183	C
Sandy-loam	15	1210 ± 92	A	2563 ± 250	A	2239 ± 103	A	6012 ± 189	A
Clay-loam	15	742 ± 20	AB	1927 ± 39	AB	1805 ± 62	AB	4475 ± 31	AB

Means within a column which do not share the same letter are significantly different ($P < 0.05$).

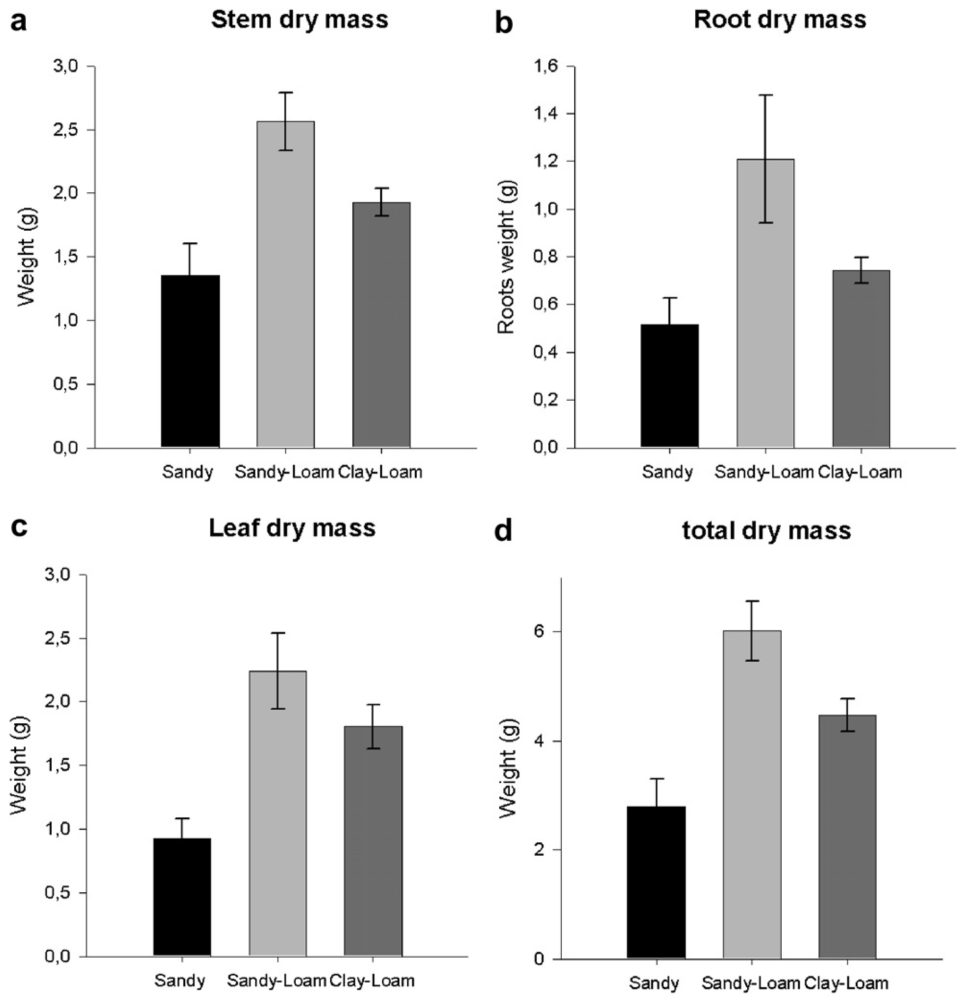

Fig. 3 – Dry mass of *Jatropha curcas* grown in the three soil types. Plants grown in Sandy-loam soil exhibited the highest dry weights compare to those located in clay-loam soil and sandy soils. Error bars represent one standard error of the mean ± S.E.

4. Conclusions

Although it is normally claimed that *J. curcas* is well adapted to marginal soils, it is quite important to denote that this study has demonstrated that *J. curcas* may not grow well in sandy soils if they are very low in nutritional contents, even though the root system shows a good potential for nutrient exploration. The results suggest that if nutritional contents are higher in clay-loam soils, these ones might be more suitable than sandy soils for plant development, and sandy-loam textures were the more appropriate to obtain good seedling development with lower nutrient contents than clay-loam textures. Based on this data it can be suggested that care must be taken when *J. curcas* plantations are planned for establishment on poor soils since these plantations may be not develop well, or at least require additional fertilization.

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