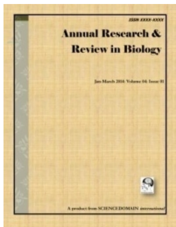

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Effects of Seed Weight and Substrate on Germination and Growth of Non-toxic *Jatropha curcas L.* Seedings

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Authors' contributions

This work was carried out in collaboration between all authors. Author OAVR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AJM managed the analyses of the study and the Figures. Author APV review and corrected the manuscript; provided de genetic material, the lab equipment and economic resources for the research. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: We characterized the responses of non-toxic seedlings of *Jatropha curcas* L. to variation in seed weight and substrate during their germination and growth.
Study Design: Two analytical experiments using a randomized controlled trial approach were applied to consider the effects of seed weight and substrate.
Place and Duration of Study: Non-toxic seeds from the east coast of Mexico were selected during two consecutive years and their seedlings were studied during one month after emergence.
Methodology: The seeds were grouped into different weight categories (400-499 - 800-899 mg). The substrates evaluated were sandy, sugarcane compost and a mix of sandy and sugarcane compost (sandy-compost, 2:1). While sandy substrate was poor in organic matter (OM), sugarcane compost was rich in OM. Germination, survival rate and mean germination time (MGT) were evaluated by seed weight category and substrate. We measured stem length, root collar diameter, number of leaves and leaf area every week,

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and at the end of the experiments total dry biomass was recorded.
Results: The heaviest seeds had higher germination rates (86.3%±7.1%) compared to the lightest seeds (69.9%±12.2%) in all substrates. Survival rate was 10% lower for the lightest seeds in sandy-compost and for the heaviest seeds in compost. MGT was not significantly different between seed weight categories or substrates (*P*=.32). Heavier seeds produced bigger seedlings in terms of stem length, root collar diameter, number of leaves, root length, and total dry mass in sandy-compost (*P*<.05). However, when substrates were compared, the substrate effect was larger than the effects from seed weight, with the largest seedlings found in compost (*P*<.05), regardless of seed weight.
Conclusion: Seed weight improved germination rates in sandy and sandy-compost substrates. Compost increased seedling growth regardless of seed weight.

Keywords: Jatropha curcas; seed weight; substrate; germination; seedling growth.

1. INTRODUCTION

The promising biofuel source *Jatropha curcas* is still under domestication [1,2]. Therefore, agronomic practices are being explored. For the Mexican non-toxic *Jatropha curcas*, scientific information is scarce. Seeds from this plant contain very low or non-detectable phorbol esters [3], the main toxic compounds widely distributed in species of the *Euphorbiaceae* [4]. Scientific research on *J. curcas* seed performance during propagation is limited [5–9]. Some field experiences in commercial plantations indicate that bigger and heavier seeds show higher viability and vigour compared with smaller and lighter seeds [10,11]. Another significant factor for seed germination and satisfactory seedling establishment in the field is the type of substrate. For *J. curcas*, studies relating germination and survival in various substrates suggest that sand can provide high germination rates [5,8,12]. However, research with other substrates and their relationships with seed weight are not available, and although there are suggestions that the plants grow well in sandy-loamy (aerated) soils; some researchers have shown that *J. curcas* could have very limited growth in poor sandy soils [13,14]. Therefore, there is a need to know the effects of seed weight and substrate on germination and growth of seedlings. The objectives of this research were to determine the effects of seed weight distribution and type of substrate on 1) Germination and survival rate, and 2) Seedling growth and biomass production of non-toxic *Jatropha curcas* seedlings.

2. MATERIALS AND METHODS

2.1 Plant Material and Seed Characterization

Native seeds from two sources along the Gulf coast of Mexico were collected. One provenance from the municipality of Medellín (18º59'52" N, 96º15'31" W; 17 masl) was collected during July 2009, and one provenance from the municipality of Alvarado (18º46'26" N, 95º45'35" W; 4 masl) was collected during August 2010. Mother plants were part of living fences without irrigation or fertilization. Both sources were located in the same geographical region, "Sotavento". The climate in the region is warm sub-humid (Aw_2, Köppen classification [15]) with minimum and maximum temperatures of 20 and 29ºC, respectively, and an annual rainfall of 1500 mm (concentrated during one rainy season from June to October) and one dry season from November to May); and the region contains regosolic soils [16]. For each collection, all ripe yellow fruits were harvested and the seeds obtained manually. The seeds were dried in a shaded and aerated place until they were completely dry. Prior to the experiment, seeds were kept inside glass bottles in a cool and dry location (18ºC).

Phorbol ester contents in the seeds were determined following [17] and did not detect any phorbol 12-myristate 13-acetate (PMA) from the Alvarado provenance, and detected only $0.19 mg \cdot g^{-1}$ from the Medellin provenance. Both provenances were considered non-toxic because local people consume cooked seeds. Seed oil content was estimated as 61% for Medellin and 54% for Alvarado.

2.2 Seed Weight Frequency Distribution and Seed Selection

All mature fruits from each selected shrub in each provenance were collected. The distribution of seed sizes was obtained by measuring seed length and width using a digital Autotec caliper (0.01 mm accuracy, range 0-150 mm) and weight on an Ohaus Pioneer PA64C analytical balance (0.0001 g precision, range 0-65 g).

2.3. Experimental Design

Two experiments were conducted: experiment 1, during May 2010, which evaluated the collection from Medellin, and experiment 2, during October 2010, which evaluated the collection from Alvarado. Based in their weight distributions and availability per collection (Fig. 1), the seed weight categories were conformed to equal seed quantities in each category. Experiment 1 consisted of 51 seeds per category and experiment 2 consisted of 32 seeds per category. Experiment 1 evaluated seed weight categories in one substrate, a mix 2:1 of sandy soil and compost made of sugarcane bagasse (obtained from a local sugar mill) and labeled as sandy-compost. Experiment 2 evaluated seed weight in two substrates: A mix of 1:1 sandy and sandy-loamy soil, labeled as Sandy; and pure sugarcane bagasse compost, labeled as compost. One seed was sown per black polyethylene bag (30 cm height and 15 cm diameter). Seeds were placed in vertical position with caruncle down and at a depth of 2 cm. The treatments were identified with permanent ink on the bag and randomly distributed under a mesh shade. Seeds and emerging seedlings were watered daily until the end of the experiments.

2.4 Experimental Conditions

The experimental site was located in the municipality of Manlio Fabio Altamirano, Veracruz, which also belongs to the Sotavento region (19º16'00" N, 96º16'32" W; 16 masl), with the same climate (Aw_2). The experimental units were placed under a mesh-shade of 25%. Plant material and site conditions registered during experiments are summarized in Table 1.

Table 1. Environmental conditions during each experiment

Experiment	Minimum average temperature ± S.D.* (ºC)	Maximum average temperature ± S.D. (ºC)	Relative humidity ± S.D. (%)
Experiment 1	23.7±1.4	34.4±1.9	74.8±5.9
Experiment 2	19.8±1.1	30.2±1.5	71.5±7.1

**S.D. = Standard deviation*

2.5 Substrate Analysis

The substrates were analyzed for pH (pH meter), nitrogen (semi-micro-Kjeldahl method), potassium (PFP7 Flame Photometer) and phosphorus (Olsen method).

2.6 Variables Measured

2.6.1 Germination

Germination was assessed from the second day until day 20 when no further germination was observed. Survival was assessed as the number of seedlings surviving after a period of 20 days following their germination.

2.6.2 Mean germination time

Mean germination time (MGT) was calculated according to [18]:

$$MGT=\sum i=1\rightarrow k(n_i t_i)/\sum i=1\rightarrow k(n_i) \quad (1)$$

Where

n_i = The number of seeds germinated only during day i; t_i = the number of days for n_i;
k = Time of the last germination event.

2.6.3 Measurements

The following variables were measured every week: shoot length (using a tape measure having 1 mm precision), measured as the distance between the root collar and the apical meristem and root collar diameter (using a digital caliper having 0.01 mm precision). At the end of the experiments, the number of leaves and the area of the first true leaf (using a tape measurement with 1 mm precision) were calculated as the product of 0.84*(leaf length*leaf width)$^{0.99}$[19]. Later, the plants were uprooted, the roots washed, and the whole plant was oven-dried at 70ºC for 72h to determine plant dry mass (using an analytical balance 0.0001 g precision). For experiment 2, additional to these variables, the fresh tap-root and four main lateral roots were measured for diameter at the base and at the tip of each root (digital caliper, 0.01mm precision) and length (tape measure, 1 mm precision). With this data the coarse root volume was estimated.

2.7 Statistical Analysis

Survival analyses were applied using the Kaplan-Meier Log-Rank test to evaluate the proportion of germination and survival seed rate in both experiments. The MGT, stem length, root collar diameter, area of the first true leaf and biomass were compared using analyses of variance (ANOVA). 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 Kruskal-Wallis test on ranks. Experiment 2, with two factors (germination and vigour by seed weight category and substrate), was analyzed using a two-way ANOVA. *Post hoc* multiple comparisons were performed using the Holm-Sidak method, at a level of significance of α=.05. Growth variables were compared weekly and at the end of the two experiments. Sigma Plot version 10.0 was used for all analyses.

3. RESULTS

3.1. Seed weight Frequency Distribution

Fig. 1 shows the frequency distribution of weight and size from the two provenances. Seeds from Medellin had a median of 623 mg and seeds from Alvarado a median of 700 mg. In both provenances, more than 68% of the weights were located in the range of 600 to 799 mg. Seed weight distribution from Medellin provided categories from 400 to 799 mg, while Alvarado provided categories from 500 to 899 mg (Table 2).

Table 2. Weight categories and their average weight, length and width (±S.D.) obtained from the provenances of Medellin and Alvarado

Experiment and provenance	Seed weight categories (mg)	Weight (mg)	Length (mm)	Width (mm)
Experiment 1	400-499	453.9±34.0	16.3±0.9	8.4±0.5
(Medellin)	500-599	545.3±21.6	16.7±0.4	8.4±0.2
	600-699	620.6±11.4	17.8±0.7	9.0±0.3
	700-799	733.8±24.8	18.6±0.6	9.2±0.3
Experiment 2	500-599	561.8±32.3	16.3±1.2	9.2±0.6
(Alvarado)	600-699	648.1±26.9	16.8±1.3	9.4±0.7
	700-799	744.5±27.9	17.6±1.2	9.7±0.5
	800-899	840.8±31.7	17.8±1.5	10.0±1.0

3.2 Substrate Analysis

Results of substrate analysis are shown in Table 3. All the substrates were located in the alkaline range, being Compost the one with the highest contents of nitrogen, phosphorus and potassium (three times more nitrogen than sandy-compost, and more than ten times nitrogen than sandy).

Table 3. Physical and chemical properties of the substrates

Substrate	pH	Nitrogen (%)	Phosphorus (ppm)	Potassium (mg/L)
Sandy-compost	7.35	0.35	20.32	81.34
Sandy	7.03	0.09	17.17	9.13
Compost	7.01	1.05	43.59	240.70

3.3 Germination and Survival by seed Weight

The proportion of the germination curves showed differences among seed mass categories (K-M Log-Rank = 185.35, P<.001), with the lightest seeds having the lowest germination rate (Fig. 2A). Survival rate was also different between categories, (K-M Log-Rank = 111.02, P<.001), with the lightest seeds having the lowest survival rate. MGT was similar independent of seed weight (H=3.9, P=.27) (Table 4).

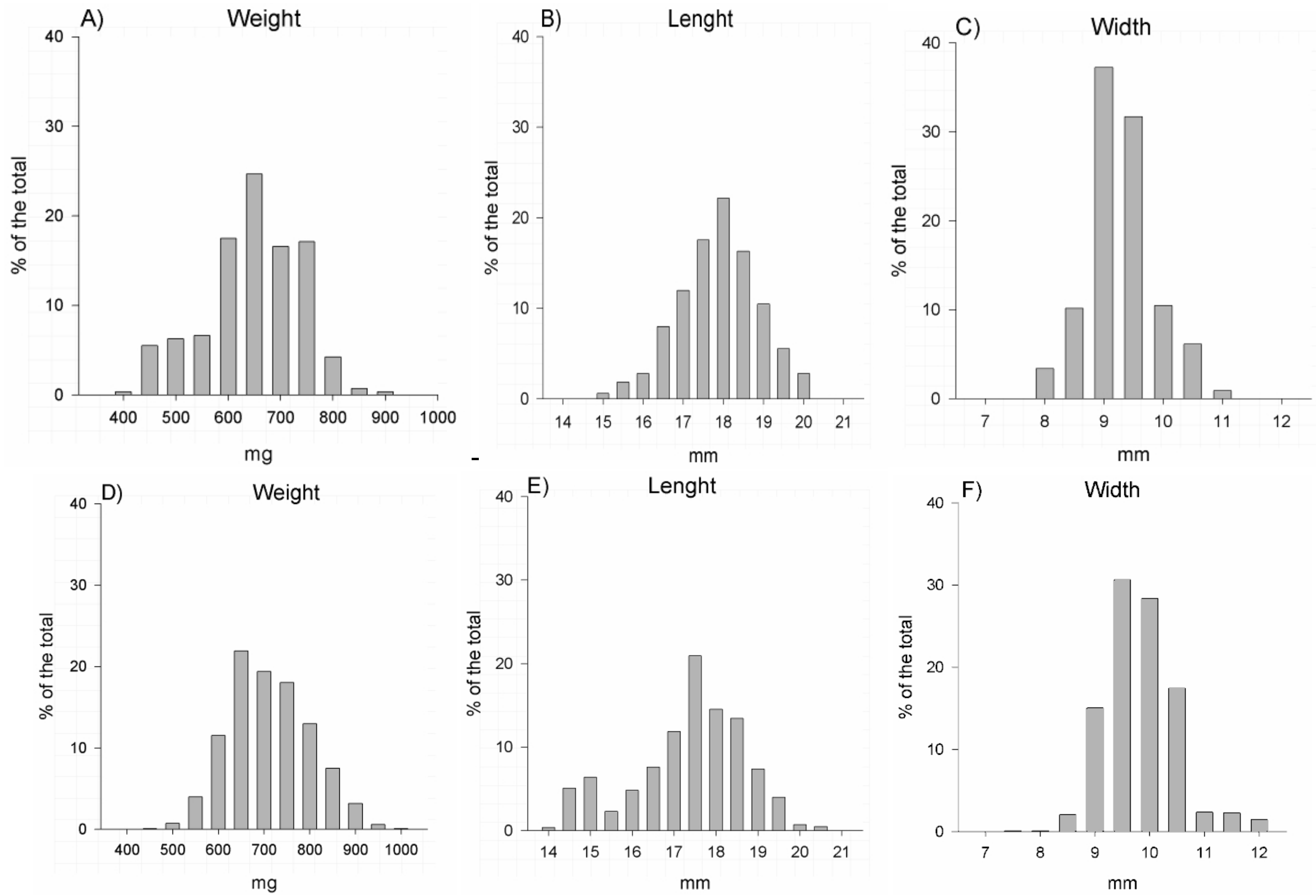

Fig. 1. Frequency distribution of seed weight, length and width from the provenances of Medellin (A, B, C) and Alvarado (D, E, F)

3.4 Germination and Survival by Substrate and Seed Weight

Germination curves differed among substrate and weight categories (K-M Log-Rank = 175.53, *P*< .001). The heaviest seeds in sandy soil had the highest germination rate, while the lightest seeds in compost had the lowest germination rate (Fig. 3A). Survival rate differed among substrates (K-M Log-Rank =17.97; *P*<.001). The heaviest seeds in compost had lower survival rate compared with categories 600-799 mg (Table 4). The MGT was similar independent of seed weight and there was no statistically significant interaction between substrate and weight (F=1.089; *P* =.36) (Table 4).

3.5 Growth Rates by Seed Weight

For experiment 1, the stem growth rate was significantly different among categories (Fig. 2B), except during the second week (H=7.23, *P*=.06) and, at the end of the third week seedlings from seeds heavier than 600 mg were taller than those of 400-599 mg (*P*<.001; Fig. 2b). Growth rate in root collar diameter was different the first week, but similar during the second and third weeks (*P*=.07). These differences, and the small weekly accumulative differences, produced wider root collar diameters in seedlings from seeds heavier than 700 mg at the end of the experiment (Fig. 2C). The number of leaves during the third week was greater in seedlings from seeds heavier than 500 mg compared with lower weights (H = 10.88; *P*=.013). Leaf area of the first true leaf was similar in the four categories (H = 3.22; *P*=0.36).

3.6 Growth Rates by Seed Weight and Substrate

Within weight categories, there were no significant differences in stem length, root collar diameter, number of leaves or area of the first true leaf (*P*=.05; Table 5). Although, during the second week the lightest category (500-599 mg) had the lowest growth rate in height.

Seedlings in compost had higher growth rates over five weeks. They also had greater stem growth rate in height and diameter since the second week and greater leaf area and number of leaves since the third week until the end of the experiment compared to seedlings in sandy soils (Figs. 3B, C). Root volumes were larger in seedlings from compost than seedlings from sandy substrate during the fifth week. However, longer roots were recorded from sandy soils.

Over five weeks there was no statistically significant interaction between weight and substrate for stem height (*P*≥.13). However, during the fourth and fifth week, there was a statistically significant interaction between weight and substrate for stem growth rate (*P*=.02), being the highest category in compost (800-899 mg) the one with the highest stem grow rates.

3.7 Dry Mass and Biomass Distribution by Seed Weight

For Experiment 1, dry root mass did not differ significantly among seedlings from different seed weight categories (Kruskal-Wallis; *P*=.06). However, stems were heavier in seedlings from seeds weighing more than 600 mg (Kruskal-Wallis; *P*<.001), and the total dry weight was higher in seedlings from seeds having weights over 700 mg (Kruskal-Wallis; *P*<.001) compared with seedlings having seed weights less than 600 mg. The greatest proportion of biomass was allocated to leaves during the third week in all seedlings (Table 6), with higher biomass in seedlings with seed weights heavier than 700 mg compared to seedlings from seeds lighter than 500 mg.

Table 4. Germination and survival rate in response to seed weight category and substrate

Experiment	Substrate	Weight category (mg)	Germination rate (%)	Survival rate (%)	Mean germination time
Experiment 1	Sandy-Compost	400-499	60.00^{b}	80.00^{b}	8.52^{a}
		500-599	98.04^{a}	88.33^{a}	7.29^{a}
		600-699	96.08^{a}	90.00^{a}	7.56^{a}
		700-799	90.20^{ab}	85.00^{ab}	7.35^{a}
Experiment 2	Sandy	500-599	62.50^{b}	90.00^{ab}	5.11^{a}
		600-699	56.25^{b}	94.40^{a}	3.72^{a}
		700-799	62.50^{b}	90.50^{ab}	5.21^{a}
		800-899	90.63^{a}	96.60^{a}	4.62^{a}
	Compost	500-599	40.63^{c}	92.00^{ab}	4.00^{a}
		600-699	46.88^{b}	100.00^{a}	4.06^{a}
		700-799	50.00^{b}	100.00^{a}	4.61^{a}
		800-899	78.13^{a}	84.00^{b}	3.65^{a}

Different letters within a column and within an experiment indicate a significant difference between treatments (P<.05)

Table 5. Variables measured (±S.D.) by seed weight category at the end of the two experiments

Experiment and treatment	Seed weight (mg)	Stem length (mm)	Root collar diameter (mm)	Number of leaves	Area of first true leaf (cm^2)
Experiment 1					
Sandy-Compost	400-499	105.2±12.9^{c}	6.3±0.5^{c}	3.6±0.5^{b}	55.1±23.1^{a}
	500-599	122.7±16.5^{b}	6.6±0.6^{bc}	3.9±0.4^{a}	60.2±17.9^{a}
	600-699	127.0±14.3^{ab}	6.8±0.7^{ab}	4.0±0.0^{a}	62.0±10.5^{a}
	700-799	135.8±20.7^{a}	7.0±0.6^{a}	4.0±0.0^{a}	64.1±9.5^{a}
Experiment 2					
Sandy	500-599	149.8±31.2^{b}	9.8±1.0^{b}	5.3±0.5^{b}	105.9±31.2^{b}
	600-699	163.7±27.9^{b}	9.8±0.7^{b}	5.6±0.5^{b}	106.1±24.5^{b}
	700-799	162.6±23.4^{b}	10.7±0.9^{b}	5.4±0.7^{b}	120.0±32.5^{b}
	800-899	152.3±26.2^{b}	11.1±1.4^{ab}	5.6±0.9^{b}	123.3±22.8^{b}
Compost	500-599	208.6±49.6^{a}	11.4±1.4^{a}	7.0±1.3^{a}	151.2±41.4^{a}
	600-699	239.4±44.7^{a}	11.1±1.3^{a}	7.2±1.7^{a}	155.8±34.9^{a}
	700-799	209.5±28.7^{a}	11.7±0.9^{a}	6.9±1.1^{a}	148.2±32.1^{a}
	800-899	236.4±50.1^{a}	11.8±1.4^{a}	7.1±1.1^{a}	149.6±36.6^{a}

Different letters within a column and within an experiment indicate a significant difference between treatments (P<.05)

3.8 Dry mass and biomass distribution by seed weight category and substrate

For experiment 2, the effects of seed weight on their seedlings were statistically similar for stems (P=.13), leaves (P=.08) and roots (P=.22). There was no statistically significant interaction between seed weight and substrate for stems (P = .43), leaves (P =.82), or roots (P=.29). However, compost produced the heaviest stems and roots (P<.001). The root to shoot ratio was greater in plants from sandy soil compared to compost (P=.01), and there was no statistically significant interaction between seed weight and substrate (P =.61).

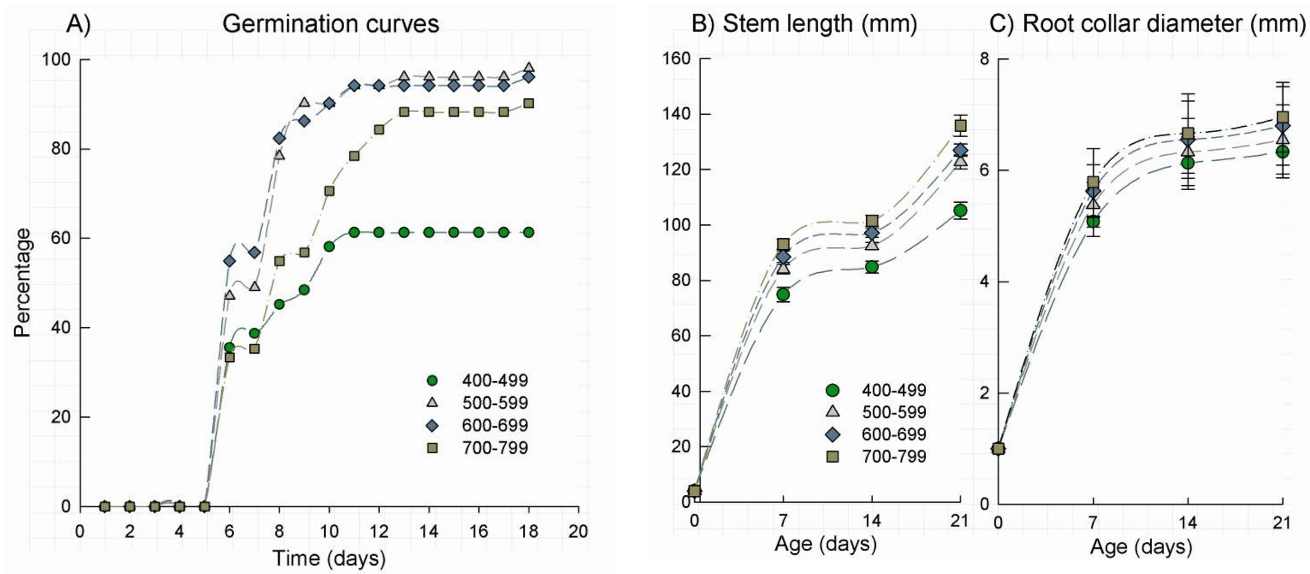

Fig. 2. Germination and growth curves from Experiment 1

Mean ± S.E.M = Mean values ± Standard error of means of four weight categories

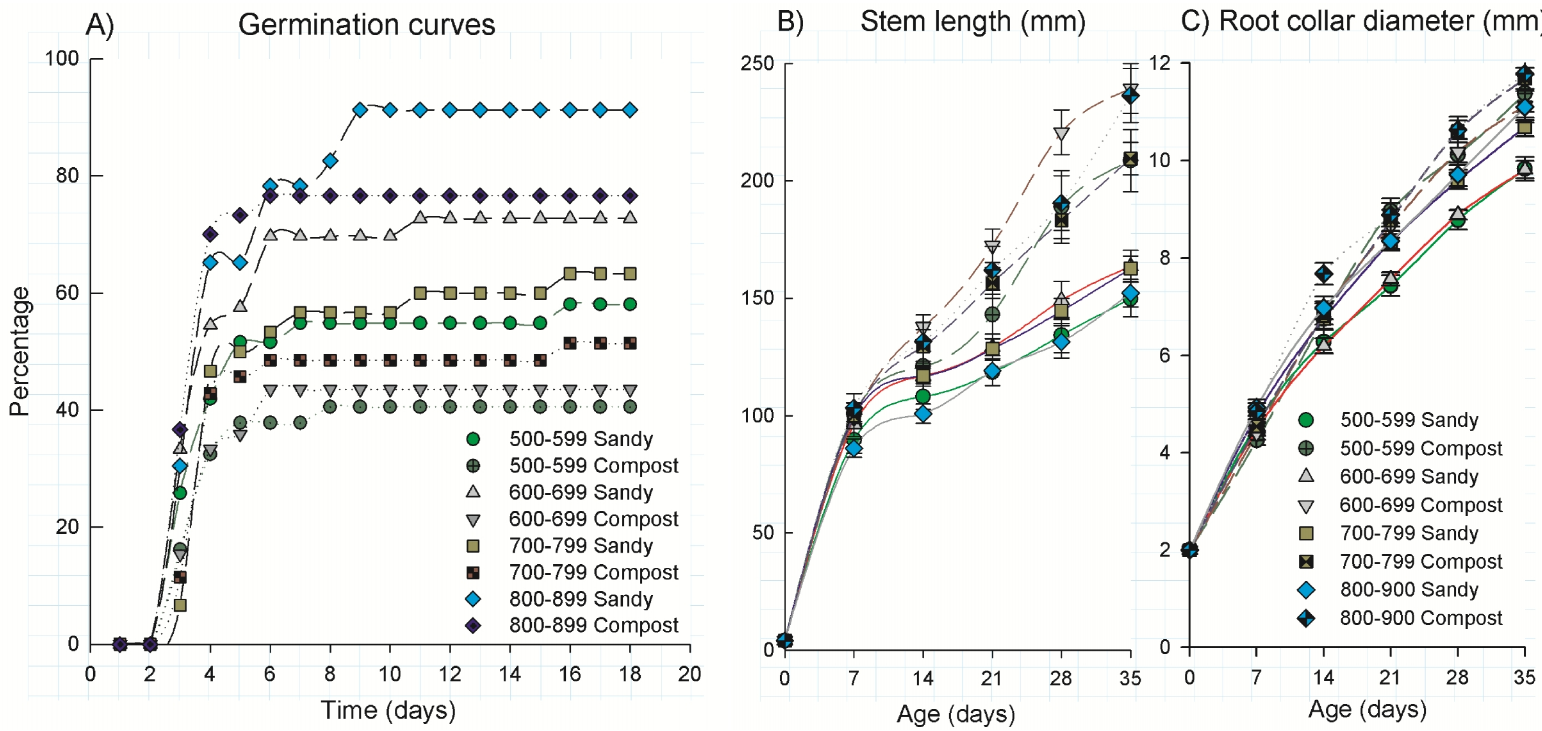

Fig. 3. Germination and growth curves from experiment 2

Mean ± S.E.M = Mean values ± Standard error of means of four weight categories

Table 6. Average dry mass and root to shoot ratio (±S.D.)

Experiment and treatment	Seed weight (mg)	Dry mass (mg)			Root to shoot ratio
		Roots	Stem	Leaves	
Experiment 1					
Sandy-Compost	400-499	236±64[a]	325±64[b]	509±116[c]	0.29±0.07[a]
	500-599	206±81[a]	341±81[b]	509±73[c]	0.25±0.07[a]
	600-699	194±105[a]	393±105[ab]	613±94[b]	0.19±0.06[b]
	700-799	218±62[a]	468±157[a]	739±118[a]	0.18±0.03[b]
Experiment 2					
Sandy	500-599	388±194[a]	2116±189[b]	1382±148[b]	0.11±0.05[a]
	600-699	373±67[a]	2214±189[b]	1472±148[b]	0.10±0.01[a]
	700-799	482±355[a]	2667±179[b]	1702±140[b]	0.12±0.11[a]
	800-899	479±184[a]	2567±174[b]	1820±136[b]	0.11±0.02[a]
Compost	500-599	438±115[a]	2953±208[a]	2215±163[a]	0.09±0.02[ab]
	600-699	453±119[a]	2826±184[a]	2357±144[a]	0.09±0.04[ab]
	700-799	405±112[a]	2902±189[ab]	2345±148[a]	0.07±0.01[b]
	800-899	498±120[a]	3201±184[a]	2511±144[a]	0.09±0.02[ab]

Different letters within a column and within an experiment indicate significant differences between treatments (P<.05)

4. DISCUSSION

4.1 Germination and Survival Rates by Seed Weight and Substrate

Seed weight influenced germination rates in *J. curcas*. Heavier seeds yielded higher germination percentages in the three substrates. These results are congruent with field experiences reported for toxic seeds of this species [10,11]. The lower germination in the lightest seeds from each provenance is also an indication that seeds with low weights at collection may not have enough maturity and energy to achieve germination [20]. Therefore, it can be considered that greater energy content in heavier seeds contributed to improve germination rates [21]. It should be noted that seeds from Medellin had 7% more oil than seeds from Alvarado and showed higher germination rates for the same weight categories (500-799 mg), although higher temperatures may also have favored this improved performance, since they were close to optimum temperatures reported for *Jatropha* [5,22].

Weight distribution also played a role in survival rate, since higher survival occurred for weights with the highest frequency of distribution (Table 4). Survival percentages according to their distribution of weights are considered a survival strategy for the most abundant seeds [23].

When compared, sandy soil was better than compost for seed germination. Better germination performance in sandy soils and soils with low organic matter have already been reported for toxic and non-toxic provenances [5,8]. Compost has a higher moisture retention and, by being rich in organic matter, favors the development of bacteria and fungi that attack seeds; while sandy soils, which are low in organic matter and provide better aeration for the seeds, favor germination [24,25].

4.2 Growth rates by Seed Weight and Substrate

In sandy-compost substrate, the heavier the seed, the larger the size of the plant resulting at the end of the third week, indicating that seed biomass affected stem growth. Seed weight also positively influenced root collar diameter in sandy, a poor organic matter substrate, with low nitrogen content. However, when soil was rich in organic matter, with higher nitrogen, phosphorus and potassium levels, the effects of seed weight were not significant. A richer substrate like compost significantly increased above and below-ground development compared to poor sandy substrates; however, the root system of *J. curcas* extended its capabilities to reach more area in this arid environment, and roots were longer in seedlings with heavier seed weights. This is an indication that seed weight positively affects the ability of *J. curcas* to survive in low-nutrient environments [26,27]. Additionally, larger and heavier seeds of *J. curcas* have been associated with better field performance and higher yield potential [6].

4.3 Biomass by Seed Weight Categories and Substrate

In sandy-compost substrate, biomass of leaves and stems was influenced by seed weight; heavier seeds produced more stems and leaf biomass. Yet, seedlings from smaller seeds had higher root: shoot ratios, compensating for the lower energy content in their cotyledons with a higher ability to explore for external resources. When seedlings germinated and grew under conditions such as with the sandy and compost media, substrates played a larger role than seed weight regarding biomass production. Thus, seedlings developing under a rich substrate will have similar biomass independently of their seed weight. The bigger root to shoot ratio in poor substrates, compared with richer substrates has been previously observed in juvenile seedlings of *Jatropha* [14]. This performance could be considered a strategy to improve foraging outcomes for soil resources, which is an adaptive survival strategy of species adapted to nutrient-poor soils [28,29].

5. CONCLUSION

There is a strong interactive effect of seed weight on germination and growth of non-toxic *J. curcas* seedlings. Heavier seeds perform better in soils with little organic matter. Substrates rich in organic matter (like compost) favor seedling growth and biomass production independently of seed weight. It is recommended to select heavier seeds for planting in soils rich in organic matter to obtain healthier and larger plants.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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