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Article

Effect of weight and size of seed on *Moringa* and *Ricinus* seedlings

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Abstract

Ricinus communis L. and *Moringa oleifera* Lam. are oleaginous with industrial applications that are promoted to be cultivated in poor soils. However, little is known about the relationship between weights and sizes of their seeds and their propagation in these media. Therefore, an investigation on these factors will allow to determine their real establishment capacities. The objectives of this study were to determine the relationship between weight and size of seeds of *Ricinus* and *Moringa* against germination and seedling growth. 80 seeds per species were randomly selected. The seeds were weighed, measured and germinated in a sandy substrate. During two months, the number of days to germinate, stem height and diameter, leaf area, robustness index and final biomass were evaluated by correlation and regression analysis with seed sizes and weights. The results for both species showed shorter germination times in lighter seeds (r= -0.27 for *Ricinus* and -0.08 for *Moringa*), but also showed a positive correlation between weights and growth variables, being higher in stems (r= 0.41 for *Ricinus* and 0.68 for *Moringa*) and for the biomass of the stems (r= 0.35 for *Ricinus* and 0.53 for *Moringa*) and the roots. The length of the seed obtained the highest correlation in stem elongation for *Ricinus* (r= 0.24), while Moringa it was the width (r= 0.61). We concluded that heavier and larger seeds will produce taller seedlings with more profuse roots in a sandy substrate.

Keywords: *Moringa oleífera*, *Ricinus communis*, diasporas, propagation, seedlings.

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Introduction

A high percentage of germination and rapid growth are key to the survival and establishment of a species, especially in arid lands, where resources are very limited; thus in these environments the plants will depend much more on the biomass of their seeds for the construction of photosynthetic tissue during their juvenile stage, Upadhaya *et al*., (2007), radher than other factors. The oil content of a seed is also important in these circumstances, since the lipid reserves are useful to achieve a constant and rapid initial growth, particularly in tropical or subtropical sites, where there is a greater competition between seedlings (Sanyal and Decocq, 2016).

Although certain oleaginous species germinate better in arid substrates, such as sandy ones, Valde-Rodriguez *et al*. (2011), once the seedlings finish their nutrient reserves, the arid environments will demand greater energy from these in order to survive and grow. In this sense, *Ricinus communis* L. (*Ricinus*) and *Moringa oleifera* Lam. (*Moringa*), both from subtropical climates, Parrotta (2009); Velazquez-Zavala *et al*. (2016), are examples of species with oilseeds that are characterized by rapid initial growth (Muhl *et al*., 2011; Nielsen *et al*., 2011). Given its importance in the pharmaceutical and energy industry, there are numerous reports on its cultivation, Severino and Auld (2013a); Zayed, (2012), but there have been no studies comparing their growth rates or their initial establishment in relation to the weight and size of their seeds on sandy substrates and without fertilization.

An analysis of their performance in these environments would allow to know their specific strategies to growth in arid or degraded sites, where species suitable for industrial uses are required that do not compete with the food species. Therefore, based on the hypothesis that the weights and sizes of the seeds will have a positive effect on greater growth and early establishment of the seedlings, the objectives of this research were to evaluate the effect of the weights and sizes of the *Ricinus communis* and *Moringa oleifera* seeds on: 1) its germination time; 2) the growth of the seedlings; and 3) the final biomass of the seedlings during their early establishment in a sandy substrate without fertilization.

Materials and methods

Selection of plant material

Seeds of *R. communis* were used from the Mexican plateau (22° 36' 12" North latitude, 100° 24' 47" West longitude), located in a backyard during a germplasm collection. The origin of the genotype could not be determined, but given its characteristics it was considered medium-sized (2.0 3.0 m tall), stem, leaf and purple cluster and fruits and grayish-purple seeds of medium size (SAGARPA, SNICS, 2014).

The seeds of *M. oleifera* were obtained from a commercial plantation located in the south central region of Mexico (18° 30' 09" North latitude 98° 35' 14" West longitude). The origin of the genotype was not provided. The mother plants had average heights between four and five meters, their pods a length between 20 and 30 cm and seeds of average size and weight, in relation to that reported for this species (Foild *et al*., 2001; Ayerza, 2011; Oloyede *et al*., 2015).

The harvest of both species took place three months before its sow. To correlate their weights and dimensions, 240 seeds randomly selected observing that they were free from defects or damage. The seeds were weighed with a digital precision scale (resolution of 0.001 g) and digital vernier measurements (resolution of 0.01 mm) on three dimensions: length (L), width (A) and thickness (E) (Figure 1). To estimate the percentage of the reserves and the embryo (almond) on the total weight of the seed, 80 seeds were extracted from the previous sample, to which the testa was removed and weighed again. The proportion of the almond was estimated as the ratio between the weight of seed without seed on the total seed weight.

Substrate, experimental design, environmental and variable conditions

The sand was obtained from a coastal dune in the state of Veracruz, Mexico. To analyze and interpret the results, the procedures of NOM-021-SEMARNAT-2000 were used. The texture, by the method AS-09 (Bouyoucos), obtained a content of sand of 96%, silt 2.5% and clay 1.5%, reason why it was considered sandy. The pH, by the AS-02 method, was 7.8 (moderately alkaline). Organic matter and N, by methods AS-07 and AS-08, were not detected, so they were considered very low. The P, by the AS-10 method, was 2.8 mg kg^{-1}, so it was considered low. Given these characteristics, the substrate was considered very low in nutrient content.

The experiment began on September 1th at the facilities of the Postgraduate College, Veracruz campus, located in the municipality of Manlio Fabio Altamirano, Veracruz (19° 11' 55" North latitude, 96° 09’ 07” West longitude, 18 meters above sea level). A completely randomized arrangement was implemented in an open-air plot, under a 50% shade mesh, with each seed-seedling as an experimental unit and 80 seeds per species, extracted from the 220 previously weighed and measured. The seeds were placed in an upright position, with a caruncle downwards for the case of *Ricinus* and with the vertical wings in the case of *Moringa* and buried approximately 1.00 cm below the surface of the substrate; which was placed in plastic bags of 20 cm deep by 15 cm wide. The bags were irrigated daily in the mornings throughout the experiment.

The variables to be evaluated were: germination day from sowing; stem length in cm (with 1 mm precision tape), stem diameter in mm (with digital vernier 0.01 mm precision), number of leaves, cotyledon length and width or first true leaf when these they were no longer present (with metric tape), in cm. With the measurements of the leaves, their leaf area was estimated according to the equations described for *Ricinus*, Naeem *et al*. (2011), and by calculating areas with scanned leaves and processed by ImageJ version 1.48 for *Moringa*.

The stems were measured every three days, to have 15 measurements at the end of the period, while the leaves were measured every seven days, with seven records during the experimental period. The average temperature of the experimental period, recorded by the meteorological station of the site, was 26.9 ±1.2 °C. At the end of the experiment the seedlings were unearthed, the lengths of their roots were measured and later the plants were separated in root, stem and leaves; weighed in fresh and placed in drying oven at 70 °C until constant temperature, to estimate dry weight. Both measurements were made using the Ohaus H-5276 analytical balance (0.001 g).

To estimate the potential content of oil in the seeds, a random sample of 20 g of seeds per species was taken, to which the husk was removed and then ground in a porcelain mortar. The oil extraction process was carried out by the Soxhlet method using 125 mL of n-hexane as solvent for 7 h.

Statistic analysis

On the measurements of the seeds their descriptive statistics were obtained (average, standard deviation, coefficient of variation (CV) and frequency distribution). To determine the relationship between seed weight and length (length, width and thickness), germination and seedling growth, the seed weight was correlated against their sizes, the number of days to germinate, the stem length and diameter, number of leaves, leaf area and robustness index (length of stem above base diameter) of the 15 samplings, as well as the fresh and dry weights of the extracted seedlings. The statistical significance of the correlations was analyzed using a two-tailed t-test with significance levels of 0.05 and 0.01. The possible relationship between seed weights and each character evaluated was estimated by testing five types of regression (linear: $y = a + bx$; exponential: $y = a e^{x}$; potential: $a x^{b}$; logarithmic: $a \ln(x) + b$; quadratic: $y = ax^2 + bx + c$), and the model with the highest coefficient of determination (r^2) was selected, as the one that could explain the greater percentage of variation, which resulted in a total of 1200 tested models per species. To evaluate correlations, models and their regressions the Sigmaplot V10.0 program was used.

Results and discussion

Distributions of seed sizes and their energy potential

For the *Ricinus* variety, both the sizes and weights of the seeds had little dispersion, with greater abundance of heavy seeds (negative asymmetry, as shown in Figure 1). The weight of the almond constituted 78% of the total weight of the seed, and its oil content was 55%. These results indicate a low variation in the seeds, when compared with studies in other *R. communis* varieties, where CVs of up to 52% were obtained for the weights (Naeem *et al*., 2011; Barrios *et al*., 2013). A low CV, predominance of high weights, high oil content and high percentage of almond on husk, are characteristic of improved genotypes (Nielsen *et al*., 2011; Barrios Gómez *et al*., 2013), so this origin could be considered an elite material for commercial use.

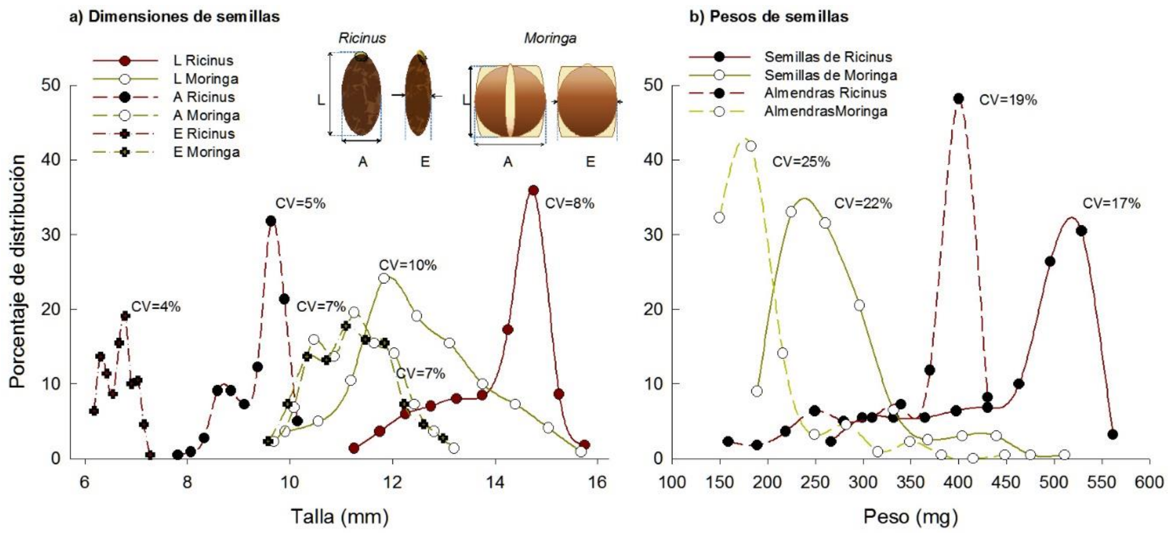


Figure 1. Distribution of sizes and weights of seeds of *Moringa oleifera* and *Ricinus communis*.

For *Moringa*, both sizes and weights were more dispersed, with a predominance of light seeds (positive asymmetry, as shown in Figure 1). The variation of the weights was found between the limits higher than those reported for African and Asian seeds, with values between 20 and 22% (Ogunsina, 2006; Ayerza, 2011), except for the distribution, which in other origins was symmetrical or it had a slight negative asymmetry (Foild *et al*., 2001; Ogunsina, 2006; Ayerza, 2011; Oloyede *et al*., 2015).

The positive asymmetry in seed weights is attributed to environmental pressures in mother plants or to a premature harvest in other tropical oilseeds (Valdes-Rodríguez *et al*., 2013; Severino and Auld, 2013b), data that the producers of this origin did not provide, but it was found out that the mother plants came from imported seeds, reason why their adaptation could generate environmental pressures that increased the dispersion and the quantity of seeds of low weight (Ayala-Cordero *et al*., 2004). The almond constituted 71% of the total weight of the seed, with 34% of oil, values similar to those of commercial genotypes in plantations in Africa, Asia and America, with percentages of oil between 30 and 42% (Olagbemide and Philip, 2014).

Relationships between weights and dimensions of seeds

In both species, the correlations between weights and seed sizes were significant (Table 1). Although only for *Ricinus* were strong, with models that managed to explain more 75% of the variation; while for *Moringa* the correlations were weak and the best models only explained about 20% of the variation, with similar results in other *Moringa* provenances from India (Ogunsina, 2006).

These values obtained are due to the shape of the cover of the seeds, which in *Ricinus* is adjusted to its almond, which generates very direct proportions with their weights; while the shape of the *Moringa* almond differs from its winged testa (Figure 2), which increases the variation in its relationships. For both species, the best regressions between weights and sizes were obtained with quadratic functions of convex curves (Table 1), which indicates that the lighter seeds had larger husks in relation to the seeds of medium weight. Although no similar analyzes were found on these two species, an analogous relationship was also found in Mexican *J. curcas* seeds (Valdes-Rodríguez *et al*., 2013).

Table 1. Coefficients of linear correlation (r) and coefficients of determination (r^2) of the regression models that obtained the best adjustments between weights, sizes and days to germinate seeds of *Ricinus communis* and *Moringa oleifera*.

Species	Weight-long		Weight-width		Weight-thickness		Weight-days to germinate	
	r	r^2	r	r^2	r	r^2	r	r^2
Ricinus	0.85^{**}	0.79^{**}	0.84^{**}	0.78^{**}	0.77^{**}	0.76^{**}	-0.27^{**}	0.11^{*}
Moringa	0.47^{**}	0.23^{**}	0.47^{**}	0.21^{**}	0.5^{**}	0.24^{**}	-0.08	0.02

*= statistically significant at 5%; **= statistically significant at 1%.

Figure 2. Cross sections of seeds of *Moringa oleifera* and *Ricinus communis* where the relationship between almonds and their heads is observed.

Germination

The seeds of *Ricinus* reached a germination percentage of 96%, with average germination time of 4.7 days, while the percentage of *Moringa* was 95%, with average germination time of 5.9 days. The correlation between the weight of the seed and the days required to germinate was negative in both species, although it was only significant for *Ricinus* (Table 1), with a potential regression model ($y= ax^{-b}$), which was significant. For *Moringa*, the regression function was quadratic, but it was not significant ($p= 0.47$). However, another species of the genus (*Moringa Peregrina*) did show a negative and significant correlation between seed weight and days to germinate, as well as another subtropical arboreal species from India (Upadhaya *et al*., 2007; Gomaa and Xavier- Pico, 2011).

In this regard, it has been recognized that in certain species the lighter seeds germinate faster in order to growth earlier and improve their life expectancies in relation to their heavier peers (Delgado *et al*., 2008). However, the climatic conditions recorded during this experiment favored rapid and high germination rates, both in *Moringa* (Tesfay *et al*., 2016) and in *Ricinus* (Ribeiro *et al*., 2015), which is why it is considered that other tests that include greater environmental stress to increase these relationships; since environmental factors can promote diverse responses in seeds, depending on their biomass or size (Cordazzo, 2002).

Seedling growth and its relation to the weight of the seed

The highest correlation between seedling weights and sizes was observed in the height of *Moringa* (r= 0.68, Figure 3), followed by the foliar area (r= 0.6), highly significant ($p< 0.001$) in both cases. The best regression was obtained with a potential model, which explained 50% of the variation in height. In *Ricinus*, the highest correlations were obtained with stem diameter (r= 0.41) and height (r= 0.4) and were highly significant ($p< 0.001$). The best regression was given by a quadratic model that predicted 21% of the diameter variation. Positive correlations, although minor (r= 0.55), between seed weight and stem length were also obtained in experiments with *Moringa Peregrina* in an arid substrate (Gomaa and Xavier Pico, 2011) and in 15-day-old *Ricinus* seedlings with a model quadratic (r^2= 0.13) (Naeem *et al*., 2011), as well as in another oilseed (*Jatropha curcas*) in sandy substrate poor in nutrients (Valdes *et al*., 2014).

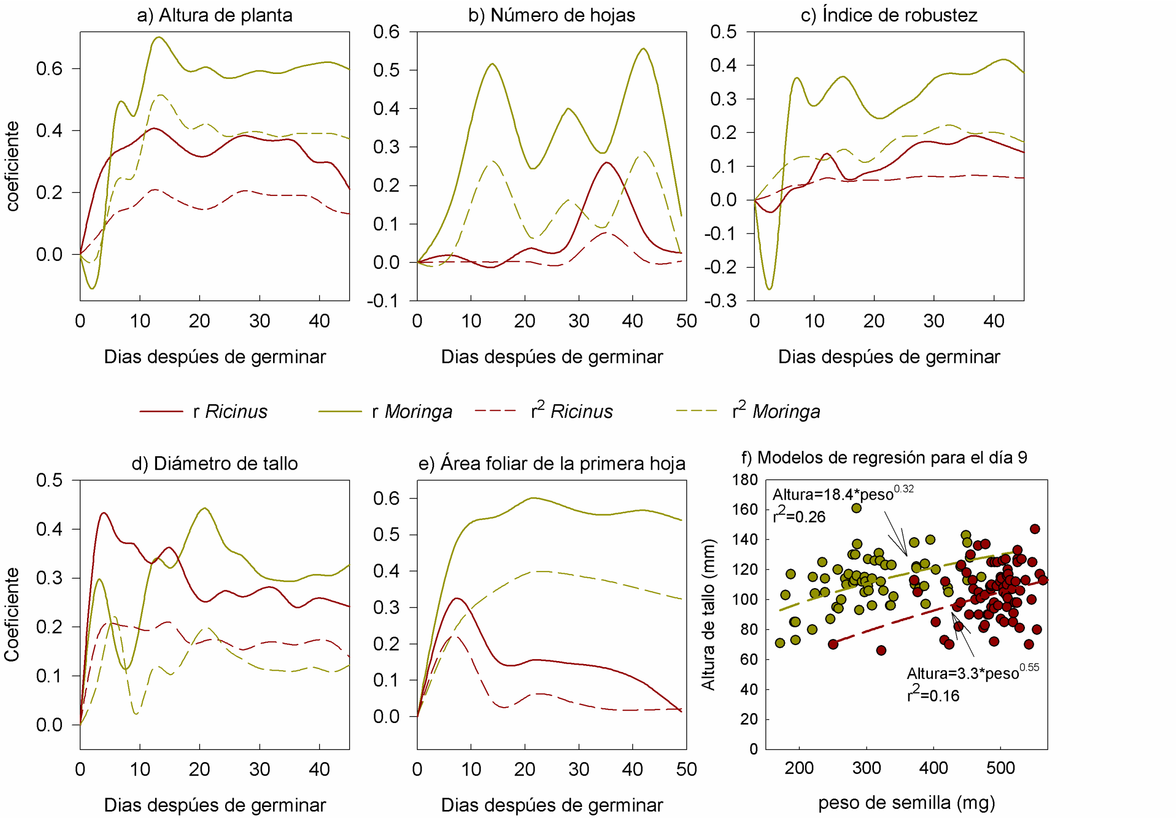


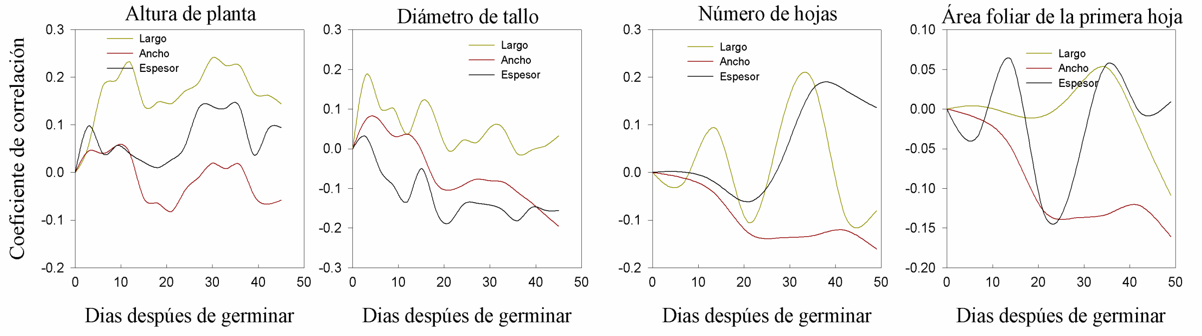
Figure 3. Correlation coefficients (r) and determination (r^2) of the models obtained between seed weights and growth variables of *Moringa oleifera* and *Ricinus communis* seedlings. Figure f) shows two examples of models obtained to define the relationship between seed weight and plant height in *Moringa* and *Ricinus* on the ninth day after germination.

The relationship with the robustness index showed no significance for *Ricinus* and had a weak correlation, although significant, in *Moringa* ($p< 0.05$), which indicates that the seedlings of heavy seeds were relatively slender. In the roots the correlations with the weights of the seeds were positive and very significant ($p< 0.01$) for *Moringa* in their maximum lengths (r= 0.42) and the diameters of their pivoting (r= 0.44), followed by the number of roots laterals (r= 0.24), while for *Ricinus* the highly significant correlations were for the diameter of its pivot (r= 0.45), the average diameter of its lateral roots (r= 0.35) and the number of laterals (r= 0.39). The thicker roots and in greater quantity in seedlings of heavier seeds imply that part of the energy resources of the seed were invested in these organs (Kabeya and Sakai, 2003), something very important in environments with limited resources, as in this sandy substrate.

Seedling growth and its relationship with seed sizes

For *Ricinus*, the highest correlation between growth and seed dimensions was obtained between seed length and stem length (r= 0.24). These results were consistent with what was reported in stems of 15-day-old *Ricinus* seedlings correlated with the size of their seeds, where r= 0.34 was reported for this relationship (Naeem *et al*., 2011), which could be explained by the fact that The axis of the embryo is directly related to the length of the seed, thus that longer seeds could develop longer stems (Figure 4 a). However, the dimensions of the seed did not seem to have significant effects on the diameter of the stem, number of leaves or leaf area. For *Moringa*, the width and the thickness of the seed were the dimensions with the highest correlations found in the length of the stem (r= 0.61 and 0.56, respectively), while the number of leaves and stem diameter had minor correlations with these measures (Figure 4 b). In this regard, no reports were found on these relationships in *Moringa*, but the fact that it was the width and the thickness of the seed the measures with the highest correlations could be due to the fact that thicker seeds have greater reserves to nourish the seedling (Fotouo-M *et al*., 2015). In both species, the dimensions of the seed that best correlated with their weight were those that had the highest correlation with the dimensions of the emerging seedling.

a) Ricinus communis



b) Moringa oleifera

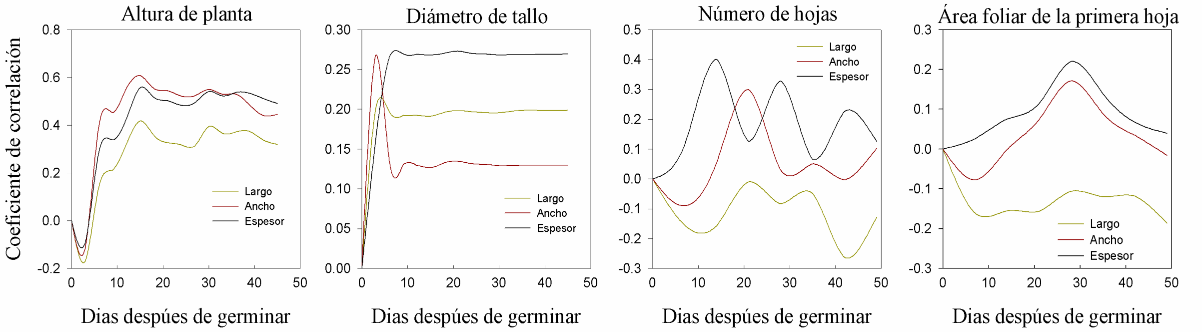


Figure 4. Correlation coefficients (r) between seed sizes and growth variables of *Moringa oleifera* and *Ricinus communis* seedlings.

The highest correlations in both weight and seed sizes obtained with *Moringa* could be due to the way in which it germinates, considered as hypogeo-cryptocotylar, where the cotyledons remain as reserves for the plant for up to 25 days after germination (Ramos *et al*., 2010; Fotouo-M.

et al., 2015), while in *Ricinus* germination is epigeous and albumen reserves are consumed when the cotyledons emerge, which are transformed into photosynthetic tissue (Severino and Auld, 2013a).

According to this strategy, it is considered that the high-energy content of *Ricinus* seeds gives them a vigorous emergence, with large leaves, taller and thicker stems, which allow them to take better advantage of solar energy to produce more foliage and rise above other plants that could compete on the same substrate (Sanyal and Decocq, 2016) (Figure 5).

On the other hand, *Moringa* emerges with thinner and smaller stems, as well as a much smaller leaf area, but the reserves of its seed allow it to maintain constant growth even in poor substrates, such as this sandy soil, where it was observed that after 27 days *Ricinus* stopped producing foliage and started its defoliation (although it was just at this stage when the heavier seedlings managed to maintain a greater number of leaves), while *Moringa* managed to maintain a 9% increase in the number of leaves until the day 42 (Figure 5 d). This indicates that *Moringa*, coming from more arid environments (Parrotta, 2009), has developed a strategy to consume the reserves of its seeds for a longer time and thus have greater opportunities to settle on less fertile substrates.

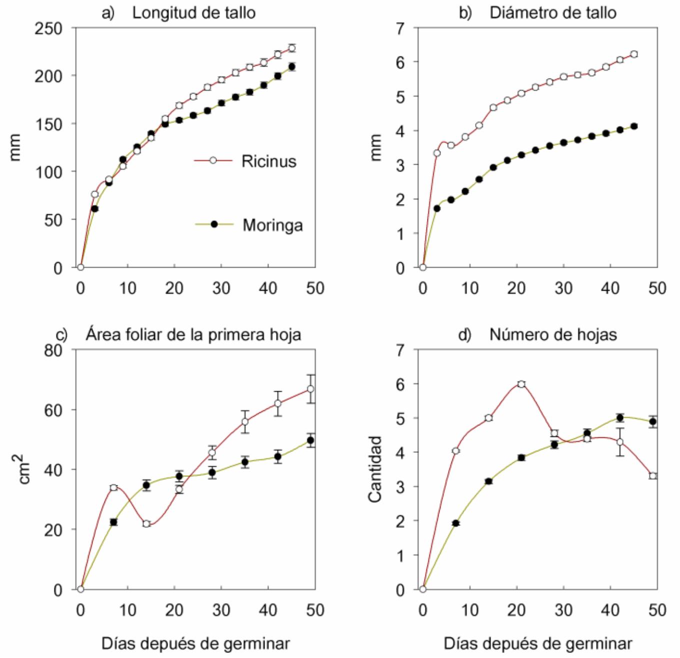


Figure 5. Stem growth curves, leaf area and number of leaves in *Ricinus* and *Moringa* seedlings.

Biomass of seedlings against seed weights

For the dry weights of the plants, the stem had the highest positive and significant correlation recorded against the weight of the seed in both species (Table 3), followed by the root and leaves. According to the regression analysis, for both species the best adjustment was obtained with a potential model, where the weight of the seeds explained 21% of the variation of the biomass of the stems of *Ricinus* and 33% of those of *Moringa*; as well as 15% of the variation of root biomass in *Ricinus* and 28% in *Moringa*. Similar results were found with other seeds of *Ricinus* and their 15-day seedlings, where a quadratic model obtained an r^2 of 0.34 (Naeem *et al*., 2011).

Table 3. Correlation coefficients and determination of the models obtained between seed weights and fresh and dry weights of 49-day seedlings.

Species	Leaves				Stems				Roots			
	Fresh		Dry		Fresh		Dry		Fresh		Dry	
	r	r^2	r	r^2	r	r^2	r	r^2	r	r^2	r	r^2
Ricinus	0.1	0.04	0.14^{*}	0.05	0.35^{**}	0.14^{*}	0.24^{**}	0.21	0.11^{*}	0.08	0.27^{*}	0.15
Moringa	0.32^{*}	0.16	0.34^{**}	0.15	0.53^{**}	0.27^{*}	0.58^{**}	0.33	0.54^{**}	0.3	0.5^{**}	0.28

*= statistically significant at 5%; **= statistically significant at 1%.

The obtained models indicate that the weight of the seeds positively influenced the final weights of the stems of their seedlings (Figure 6). Although the lower predictive capacity of the models for *Ricinus* was due to the high variation in the biomass of their plants, which at 49 days were completely autotrophic, while in *Moringa* some seeds were still found adhered to the base of the stems, which implies that their reserves could have been consuming for more than 25 days (Ramos *et al*., 2010).

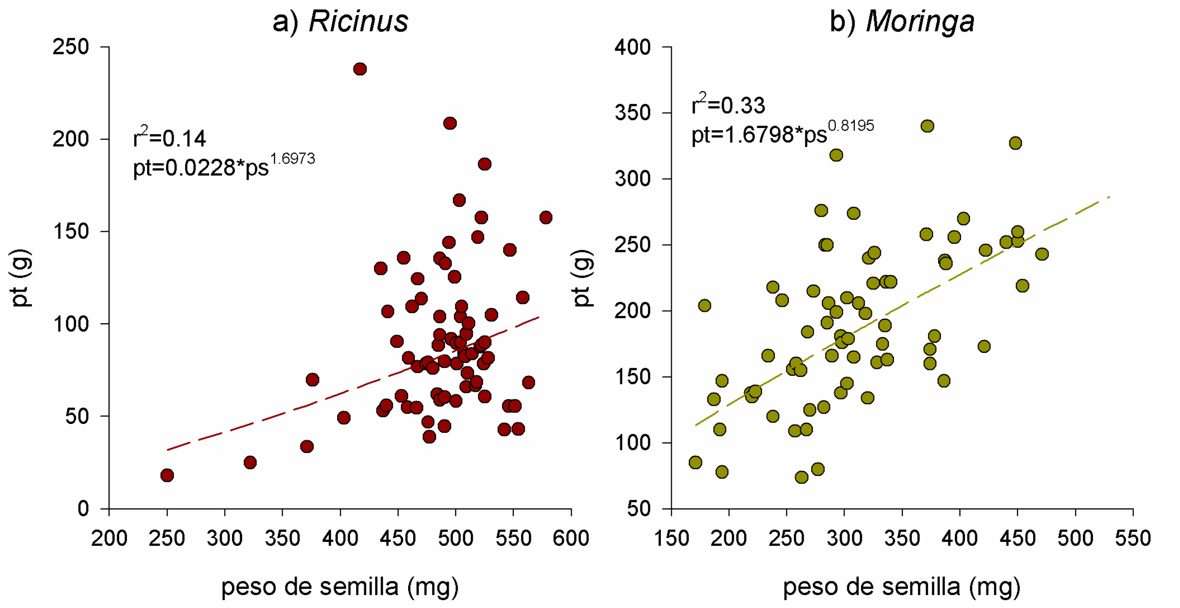


Figure 6. Models obtained to relate weight of seeds (ps) against dry weight of stems (pt) of *Moringa* and *Ricinus* seedlings.

In relation to the organs, another investigation with recently emerged seedlings of *Q. crispula* indicated that the reserves of their seed are also transported to the roots to be maintained in case the stems are eaten by predators (Kabeya and Sakai, 2003). Therefore, it can be considered that the biomass of these seeds had more impact on the stems, thus that the plants achieved higher heights and could compete better for light while more profuse roots ensure a better survival by exploring more widely a medium environment poor in resources, such as the substrate where they were.

Conclusions

The weight and dimensions of the *Moringa oleifera* seeds had a positive and highly significant effect on the growth of their stems, foliar area and roots, which was maintained for 49 days, given its hypogeal germination. While in *Ricinus* the effects of the weight and sizes of their seeds also had a positive effect on these variables, but in a lower proportion and time, given their epigeal germination; thus, although both species will propagate better with heavy and large seeds, *Moringa* will have the best advantages in a poor sandy substrate.

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