Scientific Paper

Pre-germination treatments in *Moringa oleifera* seeds and their effect on agronomic variables

Joan José Montilla-Mota[1], Weismar Grabiela Amundaray[1], Carlos Estiven Gutiérrez[2], Hadid Gizeh Fernández-Jiménez[1] and Rafael Antonio Jiménez-Francisco[1]

[1]*Instituto Nacional de Investigaciones Agrícolas, Centro Nacional de Investigaciones Agrícolas Avenida Casanova Godoy cruce con Avenida 19 de Abril, zona universitaria, vía El Limón, Maracay, Apdo. postal 4603, CP 2105, Estado Aragua, Venezuela*
[2] *Universidad Nacional Experimental Rómulo Gallegos, Guárico, Venezuela*

Abstract

The trial was conducted in the seed quality laboratory of SENASEM, in order to evaluate in seeds from *Moringa oleifera* Lamarck provenance Supergenius the effect of different hydration times (HT): 3 hours (T1), 6 hours (T2), 12 hours (T3) and 24 hours (T4), on germination (G), plant height (H) and growth rate (GR) 5, 7, 9, 12 and 14 days after sowing (DAS); as well as their effect on foliage weight (FW), root weight (RW), average of daily foliage weight (AFW) and total plant weight (TPW) 14 DAS. Trays that contained inert sand as substrate, which were placed in germination chambers, were used. The design was completely randomized blocks, with four repetitions. The variance analysis was done per variable for each evaluation date, and for the comparison of means Tukey's test was used for 5 % of significance. The results only allowed to detect significant differences for germination (5 DAS) and for H and GR (6 and 12 DAS). The hydration of *M. oleifera* seeds during 12 or 24 hours can contribute to improve the performance of the growth variables of this species. On the other hand, the RW and FW contributed in 14 and 86 %, respectively, in the total weight of the seedlings 14 DAS, which indicates that this plant can be very efficient for forage production during its initial growth stage.

Keywords: growth, germination, physiological variables

Introduction

For millennia, practically all parts of *Moringa oleifera* have been used by man. The leaves, flowers, fruits and roots are appreciated for their nutritional value and can be used feeding humans and animals. The leaves are exceptionally rich in vitamins and in different aminoacids (Fuglie, 2001). The analysis made indicate that the meal of *M. oleifera* leaves favorably compares to powder milk regarding its protein and calcium components, and additionally has a high content of vitamin A. Besides having high nutritional value, the leaves are rich in antioxidants, among which isothiocyanates stand out, which can show anti-cancerous, hypotensor, hypoglycemic and antibiotic properties. The concentrations of antinutritional factors in the leaves, such as protease, tannin, saponin and lectin inhibitors are insignificant (Olson Fahey, 2011). The leaves are also used as forage, biopesticide and for biogas production (Fahey, 2005).

M. oleifera arrived in Venezuela a little more than a century ago. It is known as the "tree of a thousand uses, marango, drumstick tree" and even "Mom's best friend", and in the Zulia state it is called "flower of guayrén or guaireña". It is an evergreen tree, of accelerated growth (Berroterán, 2015a), which usually reaches from 10 to 12 meters of height. It has an open and expanded crown, of inclined and fragile branches. The stem has a thick and whitish bark, of cork-like aspect. These characteristics make it an excellent living post and thus, splendid for ornamentation; and it also has the peculiarity of being rich in nectar and pollen, for which it is melliferous par excellence; that is, it is preferred by bees for producing honey and other derived products (Berroterán, 2015a).

The Ministry of the Popular Power for Agriculture and Lands and the Ministry of the Popular Power for Science, Technology and Innovation of the country, through the «National project for the production and use of *Moringa oleifera*» and in collaboration with the Republic of Cuba, in 2012 a program of research and production of this plant started. At the beginning of the project 286,5 ha were established in the socialist enterprises of irrigation and in the research and social production farms ascribed to both ministries, under different agroclimatic conditions of the national territory. The goal for the production of *M. oleifera* seed in 2012 was to sow 300 ha (Baldizán, 2014; Berroterán, 2015a).

Due to the importance that the national Government has recently assigned to this crop, little research generated in the country about this topic and the need to search for alternatives for animal feeding, the objective of this study was to evaluate the effect of different hydration times in *Moringa oleifera* seeds provenance Supergenius on the morpho-physiological variables of this plant.

Materials and Methods

Description of the site and experimental conditions. The trial was conducted during July, 2015, in the laboratory of seed quality control of the National Seed Service (SENASEM for its initials in Spanish), Aragua site. The seeds were obtained from the lots established for the production of such input, in the socialist production farm Los Tamarindos, located in the Zamora municipality of the Aragua State.

Experimental procedure. *M. oleifera* seeds provenance Supergenius were used, harvested in a period no longer than two months. They were extracted from mature capsules from different plants of the same cultivar, and were selected from the portion of the middle third, which had adequate moisture content, those free from mechanical damage by pathogens or insects and the ones that had the characteristic color they show when they have reached physiological maturity, according to the reports by Bayé-Niwah and Mapongmetsem (2014).

The trays that contained the samples to be evaluated were placed in metallic shelves at the same height (1 m), inside a germination chamber at constant temperature of 28 ºC, relative humidity of 50 % and with 8 hours of light per day (white light).

Measurements. The seeds were initially subject to the following treatments: T1: 3 hours of soaking before sowing, T2: 6 hours of soaking before sowing, T3: 12 hours before sowing, T4: 24 hours of soaking before sowing; for such purpose 100 seeds were introduced in glass flasks (previously sterilized), to which distilled water was added until being fully covered. In this stage those that were observed floating in each flask were removed, to avoid the use of possibly empty seeds. The seeds that would remain 24 hours in water were first soaked, and then the ones of 12, 6 and 3 hours, in order to guarantee that they were all simultaneously removed from soaking. Once this process was finished, 100 seeds were sown per germination tray (four repetitions) for each soaking time (a total of 16 trays), to which a substrate of washed and sieve inert sand had been previously added, with a 2-mm mesh, and they were later placed in germination chambers (ISTA, 2005).

At 5, 7, 9, 12 and 14 days after sowing, the germination and height reached by the seedlings were evaluated. In the case of germination, the seeds that showed visible emission of the seedling were counted; while the height was measured plant by plant from the neck or cotyledonal node to the apical bud, with a plastic graduated ruler. For each day of evaluation the growth rate was estimated, as the average difference between the heights recorded at different measurement days and the number of days corresponding to the studied period, according to the criterion expressed by Váldes-Rodríguez *et al*. (2014).

After 14 days all the plants of each experimental unit were taken, separating independently the roots (without substrate traces) and the aerial part, to dry them in stove during 24 hours. Once dry, they were weighed on an analytical scale to obtain the weight values of the foliage and the roots; and, in turn, to estimate with them the total weight (on dry basis) of the plant, through the addition of both values. Likewise, the percentage proportion of foliage weight/total dry weight and root weight/total plant weight were estimated, as well as the average daily weight of the foliage and total plant weight, by dividing the foliage weight and total plant weight between the number of days.

Data analysis. Variance analysis (ANOVA) was made according to simple classification linear model, considering the evaluation date and the means were compared through Tukey's test for 5 % of significance, after verifying that they fulfilled the normal distribution and variance homogeneity adjustment. All the analyses were made through the statistical program INFOSTAT 2016e®.

Results and Discussion

Table 1 shows significant differences for the variable germination from 5 DAS; the seeds subject to the hydration times of 12 and 24 hours had the highest percentages (26 and 28 %, respectively). Nevertheless, there was an increase in this variable at 7 days, with values that exceeded between 3,3 and 15,5 times the recorded ones for that date. In this regard, Bayé-Niwah and Mapongmetsem (2014) reported initial values of germination at 6 days, with an increase of double or triple the growth rate at 10 days depending on the position of the seed

Table 1. Average values of the evaluated variables in *M. oleifera* seedlings, subject to different hydration times.

DAS	HT (hours)	H (cm)	G (%)	GR (cm/día)	DAS	ST (hours)	H (cm)	G (%)	GR (cm/day)
5	3	1,35	9,00 ^{b}	0,27	7	3	3,83 ^{ab}	87,00	0,55 ^{ab}
5	6	1,47	6,00 ^{b}	0,29	7	6	3,61 ^{b}	93,00	0,52 ^{b}
5	12	1,25	28,00 ^{a}	0,25	7	12	5,05 ^{a}	94,00	0,72 ^{a}
5	24	1,23	26,00 ^{a}	0,25	7	24	4,98 ^{a}	94,00	0,71 ^{a}
General mean		1,33	17,13	0,27	General mean		4,37	92,00	0,63
SE		0,103	44,916	0,004	SE		0,333	15,666	0,006
9	3	9,82	94,00	1,09	12	3	19,83 ^{b}	96,00	1,65 ^{b}
9	6	9,47	96,00	1,05	12	6	21,74 ^{ab}	96,00	1,78 ^{ab}
9	12	10,26	95,00	1,14	12	12	21,11 ^{ab}	97,00	1,76 ^{ab}
9	24	10,02	96,00	1,11	12	24	22,53 ^{a}	99,00	1,88 ^{a}
General mean		9,89	95,25	1,10	General mean		21,30	96,75	1,77
SE		0,206	4,472	0,002	SE		1,040	3,444	0,007

DAS	HT (hours)	H (cm)	G (%)	GR (cm/day)	FW (g/day)	AFW (g)	RW (g)	TW (g)	TAW (g)
14	3	21,78	97,00	1,56	7,26	0,52	1,16	8,42	0,60
14	6	24,00	99,00	1,71	8,62	0,62	1,31	9,93	0,71
14	12	23,27	96,00	1,66	8,01	0,57	1,53	9,54	0,68
14	24	24,81	98,00	1,77	8,38	0,60	1,23	9,61	0,69
General mean		23,47	99,00	1,68	8,07	0,58	1,31	9,38	0,67
SE		2,252	2,888	0,011	0,452	0,002	0,091	0,671	0,003

DAS: days after sowing, HT: hydration time, H: height, G: germination, GR: growth rate, FW: foliage weight, AFW: average foliage daily weight, RW: root weight, TW: total weight, TAW: total average daily weight. a, b: values with different superscripts in each row differ at $p < 0,05$ (Tukey's test)

in the capsule, as well as a decrease in such rate at 18 days.

After the seventh day no statistical differences were found for the above-mentioned indicators, which reached values between 94 and 98 %. These results are higher than the ones obtained by Padilla *et al*. (2012), who reported 86 % of germination between 11 and 15 days when evaluating different hydration times. On the other hand, Berroterán (2015b) obtained 90 % when using seeds from the same provenance that was evaluated in this study.

For the case of the average height and growth rate values, there were only significant differences at 7 and 12 days. In this regard, Parviainen (1981) indicates that height is one of the oldest variables in the classification and selection of seedlings in nursery and is considered important for the evaluation. Likewise, Gomes *et al*. (2002) state that the height of the aerial part, when evaluated in isolation, is a variable that allows to combine it with others such as stem diameter of the root neck and the root weight/aerial part weight ratio, to have a complete estimation of the growth factors.

The height values in this research 14 DAS turned out to be higher than the ones found by Padilla *et al*. (2012) and Váldes-Rodríguez *et al*. (2014) at 40 days. Likewise, the results were better than the ones obtained by Bayé-Niwah and Mapongmetsem (2014), for the height as well as for the estimates of growth rate (from their data), which could have been due to the fact that these authors used seeds from naturalized cultivars, collected in eight localities of the Sudan-Saheila region; while in this study seed from a provenance of *M. oleifera*, from India (Toral *et al*., 2013) and taken to Cuba through the Government (Berroterán, 2015b). on the other hand, the growth values on the seventh day for the hydration times of 3 and 6 hours are close to the maximum values obtained for this variable by Pérez *et al*. (2010); however, those obtained at 12 and 24 hours are similar

to those reported by Toral *et al*. (2013) for most of the accessions evaluated at 10 days.

Figure 1 shows the average height and growth rate values which were recorded 14 DAS. For the former variable they oscillated between 21,78 (at 3 hours of hydration) and 24,81 cm (at 24 hours); and for the latter, between 1,56 (3 hours) and 1,77 cm/day (24 hours).

The highest estimated values of the daily growth rate were 1,88; 1,78 and 1,76 cm day^{-1} (for hydration times of 6, 12 and 24 hours, respectively) at 12 DAS, but after that date such indicator showed a slight decrease. On the other hand, at 5, 7, 9, 12 and 14 days the mean height values were 1,33; 4,37; 9,81; 21,30 and 23,47 cm; while for the growth rate they were 0,27; 0,63; 1,10; 1,77 and 1,68 cm day^{-1}, respectively. Both variables showed a higher slope in the growth rate of the plants at 9 days (figure 2).

For the variables foliage weight, root weight and total plant weight at 14 DAS no significant differences were found; however the lowest value was recorded for the treatment in which the seeds were

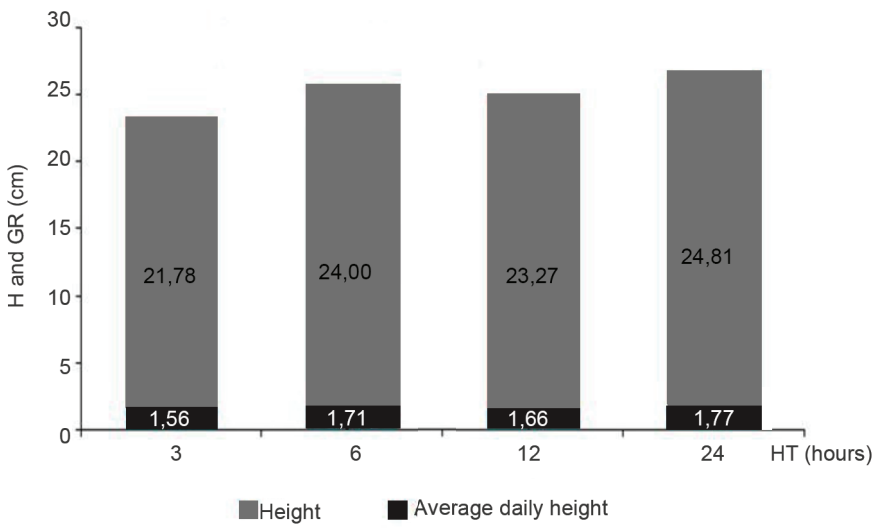

Figure 1. Height and average daily growth 14 DAS, in *M. oleifera* plants.

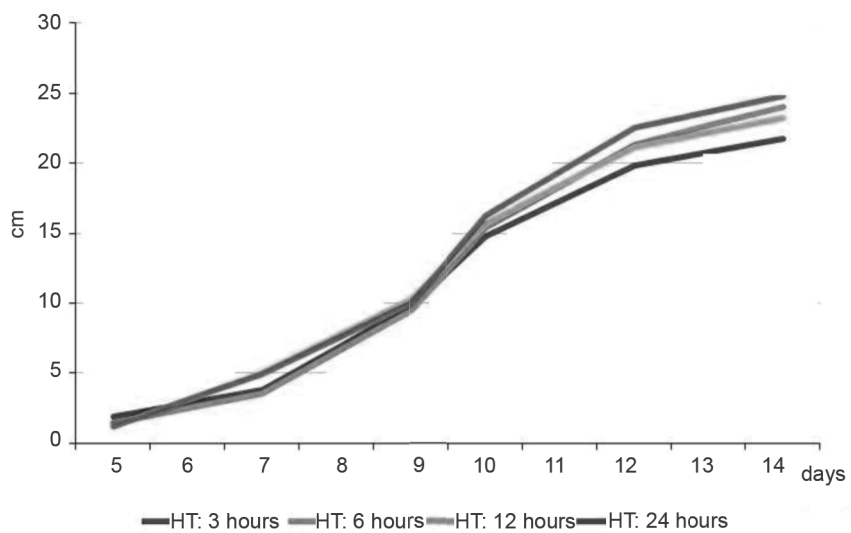

Figure 2. Growth curve of the *M. oleifera* plants.

subject to the lowest hydration time before seeding (3 hours); while the average means of these variables for the hydration time of 6 hours were equal or higher than the general means. Figure 3 shows the performance of these variables at the different hydration times at 14 DAS.

The estimated values for the daily forage weight from the total average weight of this indicator oscillated between 0,60 (3 hours of hydration) and 0,71 g day^{-1} (6 hours), with a general mean of 0,67. In this sense, Peil and Gálvez (2005) stated that the distribution of dry matter between the roots and the aerial part of the plats can be described as a functional balance between the activity of the root system (absorption of water and nutrients) and that of the aerial part (photosynthesis), because the relation between the root mass and the aerial part mass is proportional to the relation between the specific activity of the aerial part and that of the roots; which contributes to increase the specific activity of the root system, just like the adequate contribution of water or macronutrients (especially nitrogen), and the increase of the water potential.

Figure 4 shows the percentage proportion of the foliage weight and the root weight, with regards to the total weight of the *M. oleifera* plant at 14 days of age.

In this regard, Peil and Gálvez (2005) indicated that the yield of a crop is given by the capacity to accumulate biomass in the organs that are aimed at harvest. The appropriate balance between photoassimilates

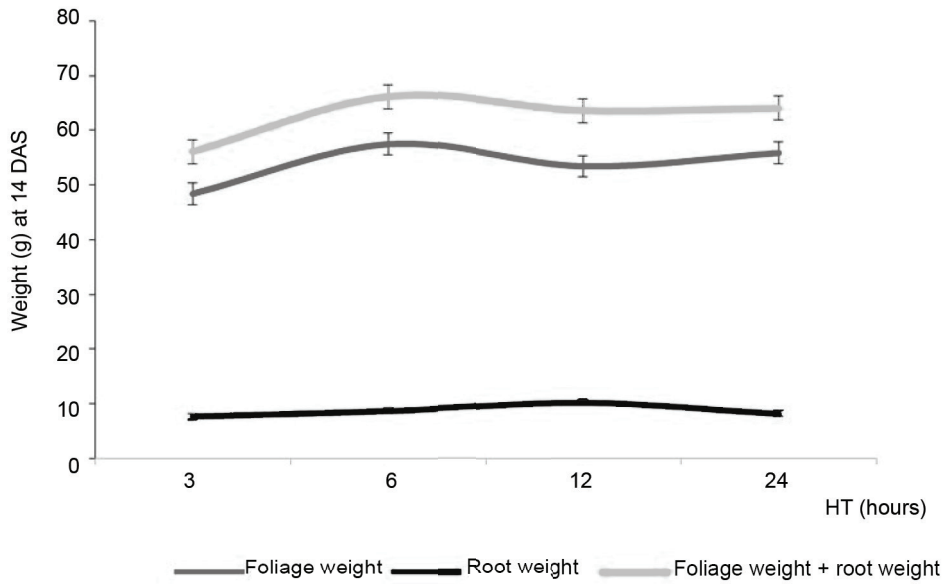

Figure 3. Weight of the *M. oleifera* plants at 14 days of age.

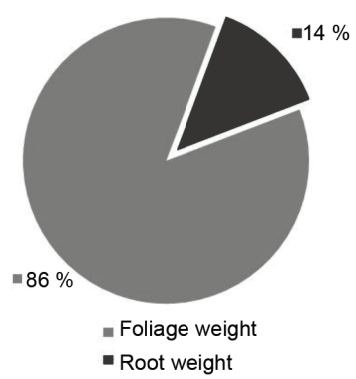

Figure 4. Proportion of the foliage weight and the root weight with regards to the total weight of *M. oleifera* plants, 14 DAS.

for the different parts of the plant is highly important to optimize production, and can be obtained through an adequate source/sink relation. Based on this and considering the obtained results, it can be stated that *M. oleifera* is an efficient plant in the production of green biomass (at least during its initial growth stage), which, along with its nutritional values ratifies it as an alternative and attractive species for forage production programs in Venezuela (especially in a plan of forage for cutting).

This statement acquires higher importance if it is taken into consideration the report by Pérez (2011), regarding the fact that the cost of feeding cattle was ten times lower when offering them *M. oleifera*, than when balanced feedstuffs were used. The digestibility (utilizable percentage of the forage consumed by a ruminant) and efficiency tests conducted with cattle showed similar feed conversion rates and milk production as the ones obtained with balanced feedstuffs and forages. In this regard, Berroterán (2015a) states that, due to the high biological value of its components, *M. oleifera* could be used as a forage of unparalleled conditions for the nutrition of beef and dairy cattle, goats, sheep, pigs, poultry and fish; as well as for the elaboration of protein meal, which is the raw material of concentrate feeds for animals, of high conversion and low cost.

Conclusions

The hydration of *M. oleifera* seeds during 12 or 24 hours can contribute to improve the performance of the growth variables of this plant. On the other hand, the root weight and foliage weight contributed in 14 and 86 %, respectively, in the total seedling weight 14 days after seeding, which indicates that this plant can be very efficient for forage production during its initial growth stage.

Bibliographic References

Baldizán, A. Moringa: el árbol milagroso. Plan nacional para la producción y uso de la moringa. Proyecto presentado al Consulado General de Venezuela en Curazao, 2014.

Bayé-Niwah, C. & Mapongmetsem, P. Seed germination and initial growth in *Moringa oleifera* Lam. 1785 (Moringaceae) in Sudano-Sahelian zone. *Int. Res. J. Plant Sci*. 5 (2):23-29, 2014.

Berroterán, J. La revolución del conocimiento: la moringa en Venezuela (III). Labranza. 3 (5):44-45, 2015a.

Berroterán, J. La revolución del conocimiento: la moringa en Venezuela y eco-socialismo (IV). *Labranza*. 3 (5):46-47, 2015b.

Fahey, J. W. Moringa oleifera: a review of the medical evidence for its nutritional, therapeutic, and prophylactic properties. Part 1. Trees for life Journal. 1 (5). http://www.tfljournal.org/article.php/20051201124931586. [18/05/2015], 2005.

Fuglie, L. J. The miracle tree: the multiple attributes of Moringa. Dakar, Senegal: Church World Service, 2001.

Gomes, J. M.; Couto, L.; Leite, H. G.; Xavier, A. & Garcia, Silvana L. R. Parâmetros morfológicos na avaliação da qualidade de mudas de *Eucalyptus grandis*. R. Árvore. 26 (6):655-664, 2002.

ISTA. International rules for seed testing. Bassersdorf, Switzerland: International Seed Testing Association, 2005.

Olson, M. E. & Fahey, J. W. *Moringa oleifera*: Un árbol multiusos para las zonas tropicales secas. Rev. Mex. Biodiv. 82 (4):1071-1082, 2011.

Padilla, C.; Fraga, N. & Suárez, M. Efecto del tiempo de remojo de las semillas de moringa (*Moringa oleífera*) en el comportamiento de la germinación y en indicadores del crecimiento de la planta. Rev. cubana Cienc. Agríc. 46 (4):419-421, 2012.

Parviainen, J. V. Qualidade e avaliação de qualidade de mudas florestais. Anais. Seminário de sementes e viveiros florestais. Curitiba, Brasil: FUPEF. p. 59-90, 1981.

Peil, Roberta M. & Galvez, J. L. Reparto de materia seca como factor determinante de la producción de las hortalizas de fruto cultivadas en invernadero. R. bras. Agrociência. 11 (1):5-11, 2005.

Pérez, R. Moringa oleífera: Una alternativa forrajera para la producción de ovinos. México: Universidad Autónoma de Sinaloa, 2011.

Pérez, Y.; Valdés, L. R. & García, F. *Moringa oleifera*. Germinación y crecimiento en vivero. Revista Ciencia y Tecnología Ganadera. 4 (1):43-47, 2010.

Toral, Odalys; Reino, J.; Santana, H. & Cerezo, Y. Caracterización morfológica de ocho procedencias de *Moringa oleifera* (Lam.) en condiciones de vivero. Pastos y Forrajes. 36 (4):409-416, 2013.

Váldes-Rodríguez, Ofelia A.; Muñoz-Gamboa, C.; Pérez-Vázquez, A. & Martínez-Pacheco, Luz E. Análisis y ajuste de curvas de crecimiento de *Moringa oleífera* Lam. en diferentes sustratos. Revista Científica Biológico Agropecuaria Tuxpan. 2 (2):66-70, 2014.

Received: April 6, 2017
Accepted: June 12, 2017