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PREGERMINATIVE TREATMENTS AND SEED MASS AS A STRATEGY TO IMPROVE SEEDLING PRODUCTION OF GUATEMALTECA AVOCADO RACE

Tratamientos pregerminativos y masa de la semilla como estrategia para mejorar la producción de plántulas de aguacate raza Guatemalteca

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ABSTRACT. Avocado crops in Colombia has many technological challenges to be a sustainable production system. Within these areas highlights the need to produce plant material with a specific genetic identity, in short time and excellent quality. The objective of this study was to evaluate different strategies for pregermination and determine the seed optimal mass of *Persea americana* var. *guatemalensis* known as race Guatemalteca in order to reduce germination times and enhance the development of seedlings in nursery stage. The variables evaluated were: germination percentage, average germination time, average speed of germination, height, percentage of viable roots, percentage of used seed reserves, biomass and leaf area. Evaluations were performed at the beginning of the experiment and after 60 and 120 days starting treatment. The design was completely randomized, with five replicates and two in time. The apical, basal, and lateral tipping seed with the seed coat removing decreases emergence time's germination in this species in 40 days. For its part the mass seed significantly affected ($p<0,05$) the uniformity and seedling development, resulting in the optimum mass was between 40 and 50 g. This paper reports the appropriate techniques for producing seedlings of avocado used as patterns under a traditional system.

Key words: sustainability, growth, germination

RESUMEN. El cultivo de aguacate en Colombia presenta muchos retos tecnológicos para lograr ser un sistema productivo sostenible. Dentro de estos aspectos se destaca la necesidad de producir material vegetal con una identidad genética determinada, en cortos periodos de tiempo y de excelente calidad. El objetivo de este trabajo fue evaluar distintas estrategias de pregerminación y determinar la masa óptima de la semilla de *Persea americana* var. *guatemalensis* conocida como raza Guatemalteca con el fin de lograr reducir los tiempos de germinación y mejorar el desarrollo de plántulas en etapa de vivero. Las variables evaluadas fueron: porcentaje de germinación, tiempo medio de germinación, velocidad media de germinación, altura, porcentaje de raíces viables, porcentaje de reservas usadas de la semilla, biomasa y área foliar. Las evaluaciones se realizaron al inicio del experimento y a los 60 y 120 días después de iniciados los tratamientos. Se utilizó un diseño completamente al azar, con cinco repeticiones y dos en el tiempo. El despunte apical, basal y lateral de la semilla junto con la eliminación de la testa, disminuye los tiempos de germinación en semillas de esta especie en 40 días. Por su parte, la masa de la semilla afecta significativamente ($p<0,05$) la uniformidad y desarrollo de las plántulas, dando lugar a que la masa óptima esté entre 40 y 50 g. Este trabajo informa técnicas para la adecuada producción de plántulas de aguacate a partir de patrones, bajo un sistema tradicional.

Palabras clave: sostenibilidad, crecimiento, germinación

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INTRODUCTION

In Colombia, avocado is planted (*Persea americana* Mill) from sea level up to 2,500 meters high, mainly for the local market. The vast majority are native species, without any process of modernization, something that has changed in the tech recent years with the planting of commercial varieties, especially in cold and moderate cold climates, this due to the increase in per capita consumption of the country and its export potential, such as fresh and processed fruit, especially Hass variety (1, 2).

The plantation success of late production such as avocado crop depends on the quality and uniformity of plant material. In this species may be present multiple problems associated with nursery phase, within which are the incompatibilities pattern glass, low germination rates, lack of identity and genetic non-uniformity, low adaptation to specific conditions and health problems (1-3). These problems are compounded because the seed used in nurseries in Colombia, a large part comes from collected fruits without quality criteria and genetic identity in collection centers, galleries and dumps farms, among others[A].

The problems associated with the production of avocado seedlings wanted solved by the technique spread by multiplication of tissues *in vitro*, which have sought to shorten the period of propagation and mass produce plants on clonal rootstocks (4, 5). However, it has achieved limited success in the tests, because this species behaves as recalcitrant *in vitro* culture, presenting many difficulties, which results in percentages very low feedback (4, 5). This situation forces to improve traditional systems propagation used as patterns, based on reproduction by seed of the various natives of *P. American* materials in the producing regions. This phenomenon of the planted area expansion in the country requires the production of plant material of excellent quality, with a particular identity, reducing production time in the nursery. Given this need this work was to evaluate different strategies to determine the optimal pre-germination and seed mass of *Persea americana* var. *guatemalensis* known as Guatemalan race, in order to achieve lower germination times and increase the uniformity of seedlings avocado used as standards. The work done in these two topics are few and very old, so should deepen their knowledge and improve existing ones.

[A] Gutiérrez, A.; Castro, D.; Ararat, M.; Melo, J.; Carvajal, S. y Ríos, D. “Diseño de estrategias para la obtención de plántulas sanas de aguacate, en condiciones de vivero”. En: III congreso Latinoamericano del Aguacate, Simposio Propagación, Medellín, Colombia, 2009, p. 39.

MATERIALS AND METHODS

Location

This research was developed in the laboratory of Tropical Crop Science and Plant Physiology and greenhouse National University of Colombia at Medellin (6° 15N, 75° 35’W, 1495 m s. n. m.). Average weather conditions in greenhouse were 20 ° and 85 % relative humidity and photosynthetically active radiation 650-1920 pmol photons m^{-2} s^{-1} on life zone called Pre-montane, Humid Forest (bh-P-M). The tests were conducted between 2012 and 2014.

Pre-germinating treatments

Selection of seeds

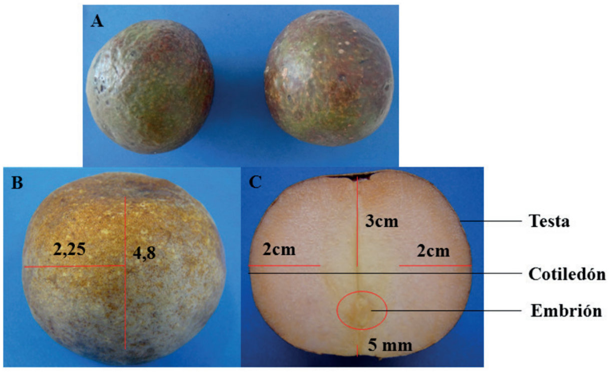
The seeds used were from healthy fruits from native trees of *P. americana* var. *guatemalensis* (Figure 1A), harvested at physiological maturity, at the discretion of 25-30 % dry matter arbitrarily assigned. This variable was determined by taking 100 g of pulp and drying oven microwave[B]. These plants are part of a commercial orchard located in the municipality of San Pedro de los Milagros in the department of Antioquia with moderate cold weather, which have been used as seed producing trees for patterns in commercial nurseries (6° 24’34, 81 “N, 75° 36’21,06” O, 2 540 m). These seeds underwent morphological characterization by evaluating parameters such as: type of adhesion of the seed to the pulp; mass; shape; long; width; color and testa size and cotyledon, as directed by Avilan and Rodriguez (6). Initially a selection of seeds was performed to obtain a healthy submit visual appearance and homogeneity in size and mass (Figure 1 B).

Surface disinfection was performed by rinsing in tap water, then washing in aqueous sodium hypochlorite (3 % v: v) for one minute, followed by washing in sterile distilled water, new immersion in aqueous alcohol (70 % v: v) and finally washing in sterile distilled water. These seeds were placed in absorbent napkins and dried at room temperature (25 °C and 70 % humidity).

Pregerminative evaluated treatments

The treatments were: T0: control, seed placed to germinate after disinfection; T1: removing the testa; T2: apical blunting seed, for which a cut of 3 cm was performed; T3: T1 + T2; T4: T2 more lateral tipping (all sides) of the seed, which was 2 cm; T5: T1 + T4; T6: basal T4 more blunting of 5 mm; T7: T1 + T6.

[B] Ochoa, S. Calidad y manejo poscosecha del fruto de aguacate. III congreso latinoamericano del Aguacate Medellín Colombia octubre del 2010, pp. 1-17.



A: visual aspect of the fruits of genotype *P. americana* evaluated
B: shape and dimensions of the discovered seed of the genotype evaluated *P. americana*
C: Parts of the seeds and diagram cuts made in pre-germinating treatments evaluated in the genotype of *P. americana*

Figure 1. Visual aspects associated with the seeds of genotype evaluated *P. americana* var. *guatemalensis*

The cuts were made with surgical scalpels, previously disinfested and with due care not to damage the embryo (Figure 1 C).

Each treatment was placed on a plastic tray germinating where the experimental units were wrapped in absorbent napkins 25 ºC, humidity 90 % and 12 hours of light and dark. Five repetitions for each treatment, where the experimental unit consisted of five seeds were evaluated.

With the data obtained the following variables were calculated: germination percentage (GP), mean germination time (MGT) and mean speed of germination (MSG)[C].

In order to determine the viability of the seeds, the tetrazolium test was performed, for which the seeds were cut longitudinally in order to remove the embryo to subsequently perform immersion in solution of chloride 2, 3, 5-triphenyl -2H-tetrazolium 0,5 % (w/v) for 24 h at 30 °C under dark conditions (7). This procedure was developed at the beginning of the trial, for which a random sample was taken, which consisted of 50 seeds. Meanwhile, at the end of the test it was applied to all experimental units. This process is completed, the embryos were observed in stereomicroscope. The viable embryos were those in which red coloration was presented and nonviable tissue in which no color was observed.

[C] Cárdenas, J. Morfología y tratamientos pregerminativos de semillas de granadilla (Passiflora ligularis Juss.). [Tesis de maestría]. Facultad de Agronomía, Universidad Nacional de Colombia, Bogotá, 2011, 110 pp.

Independent of the previous stage for this genotype evaluated germination and seed viability over time was determined, for which 12 evaluation periods where each period consisted of 1,5 months for a total of 18 months selected. In each 10 seeds, which called for above zero periods were from sawdust storage in sealed cardboard boxes fully placed in ambient conditions (20 °C temperature and 60 % humidity) they were used.

Seed mass

Six different treatments equivalent mass of the seed were evaluated: T1 => 60 g, T2 = 60-50 g, T3=50-40 g, T4 = 40-30 g, T5 = 30-20 g and T6 = <20 g.

Processed seeds were sown in plastic pots (1 kg of dry soil capacity) with substrate (50 % soil, 25 % rice husks, 25 % humus), previously sterilized by autoclaving at 103 351,5 Pa and 121°C, for two hour cycles. The plants were kept in conditions of home-mesh, fertilized with Hoogland nutrient solution consisting on the following concentrations (mg L^{-1}): nitrogen N: 50 (KNO_3); potassium K: 132 (KNO_3); phosphorus P: 160 ($NH_4H_2PO_4$); calcium Ca: 120 ($Ca(NO_3)_2$); magnesium Mg: 106 ($MgSO_4$); sulphur S: 204 ($MgSO_4$); zinc Zn: 10 ($ZnSO_4$); copper Cu 5 ($CuSO_4$); boron B: 0,8 (H_3BO_3); manganese Mn: 1,81 ($MnCl_2$) and molybdenum Mo: 0,5 (H_2MoO_4).

This solution was applied to the soil in a given amount as the daily requirement in order to maintain soil moisture to 50 % of the maximum capacity of moisture retention, given evaluation by saturating the substrate and quantitated from soil wet and dry in an oven.

The treatment was divided into three parts, the first after germination, the second at 60 days after germination (DAG) and the third at 120 DAG. The variables evaluated were: the percentage of germination; the height of the seedlings, (which was determined by a digital measuring calliper (Mitutoyo Digimatic Caliper ®), from the bottom of the stem, located on the substrate until the last apical bud developed); the percentage of viable roots (8); mass air dry and of root, to which all parts of the plants were collected, they were packed in paper bags and were carried stove (Binder®) to and dried at 70 °C until presented constant weight; leaf area using a leaf area meter Li-Cor® LI 3000A and percentage of seed stocks; for determining the latter a sample from each seed, which was dried in Semi-automatic oven (Binder ®) at 70 °C for 48 h and weighed separately cotyledons and pericarp was obtained.

With these data, regressions for each treatment were calculated in order to estimate the dry mass of cotyledons it is how the reserves used were calculated as the difference between the dry mass of the initial cotyledon and remaining after 60 and 120 DAG.

The reserve use percentage multiplied by one hundred was calculated as the ratio of reserves used and the dry mass of initial cotyledon hundredfold (9, 10). The experimental unit consisted of three seeds and germination after two seedlings. For each treatment were performed five repetitions.

Statistical analysis

For all experiments was used completely random design with two repetitions over time. Homoscedasticity and normality of the data was analyzed (p <0.05) using the criteria Levene and Kolmogorov-Smirnov, respectively (11, 12). Variance analysis and comparison test of Tukey (p <0.05) (13) was performed. For their part in the analysis of the characteristics of seed germination and viability over time the data were analyzed for the mean and standard deviation respectively.

RESULTS AND DISCUSSION

Biometric characteristics of seeds

The seeds of genotype *P. americana* var. *guatemalensis* evaluated was attached type, whose mass was quite variable with a mean value of 0,040 ± 0,0258 kg, density of 2* 10^{-4} kg m^{-3} ± 0,0001, oblate shape type, 0,048 m ± 0,04 height, diameter 0,022 m ± 0,03, 47,06 % of average humidity ± 10. Testa of color light brown 1 * 10^{-4} m thick, hemispherical, smooth and creamy cotyledons (Figure 1 B and C).

Pre germinative treatments

For parameters, germination percentage and seed viability no significant differences (p> 0.05) occurred in any of the treatments evaluated, where the first variable ranged between 80 and 88 %. Concerning this viability it was 100 %, indicating that the selected seeds were viable for each of the treatments and that none of these affected this variable (Table).

These results indicate that none of the pre-germination treatments increases the percentage of seed germination evaluated genotype avocado and identifying its initial viability was 100 %, there is some latency not eliminated with the strategies evaluated. In this regard it is proposed that low levels of germination in *P. americana* are because this plant species presents inhibition in germination, but still the type and the mechanisms involved in this process is unknown (1). Contrary to what was found in this work is reported that treatments associated with the removal of the seed coat and cotyledons cuts to improve germination rates of 12,2 to 92,2 % (14).

Meanwhile the GMT variable was grouped by Tukey test in four different groups, in the first T1 treatment, in which a decrease of GMT (p <0.05) was found compared to the control (T0) it found but lower (p <0.05) than the other treatments. In a second group treatments T2, T3, T4 and T5, which achieve a significant (p <0.05) GMT, with respect to control (T0) and T1 gathered treatment. In the third group that are the T6 and T7 treatments, the largest decrease in time needed for a seed germinates avocado evaluated genotype is achieved. The last group is formed by the (T0) control (Table).

Table. Effect of different pre-germinative treatments on of evaluated genotype *P. americana* var. *guatemalensis* seed

Treatment	Germination (%)	TMG (days)	VMG (days)	Viability (%)
T0	80 ± 8,9 ns	55,3 a ± 4,5*	1,1 b ± 0,5*	100 ± 0,0 ns
T1	84 ± 4,8 ns	35,3 b ± 3,2**	1,2 b ± 0,4*	100 ± 0,0 ns
T2	83,9 ± 5,1 ns	30,2 c ± 2,3**	1,3 b ± 0,3*	100 ± 0,0 ns
T3	87 ± 4,3 ns	27,9 c ± 2,1**	1,3 b ± 0,2*	100 ± 0,0 ns
T4	85 ± 6,3 ns	24,5 c ± 4,3**	1,4 b ± 0,3*	100 ± 0,0 ns
T5	82 ± 8,7 ns	22,3 c ± 2,2**	1,4 b ± 0,34*	100 ± 0,0 ns
T6	88 ± 6,9 ns	18,2 d ± 1,8***	1,9 a ± 0,31**	100 ± 0,0 ns
T7	8,3 ± 3,9 ns	15,2 d ± 3,6***	2,1 a ± 0,48**	100 ± 0,0 ns

Values represent mean for each treatment, followed by the standard error of the mean standard error
ns: means that no significant differences were presented in the analysis of variance (p>0,05)
* Denotes statistical difference and different letters and that the means are different, according to Tukey test (p<0,05)
MGT (mean germination time)
MSG (mean speed of germination)

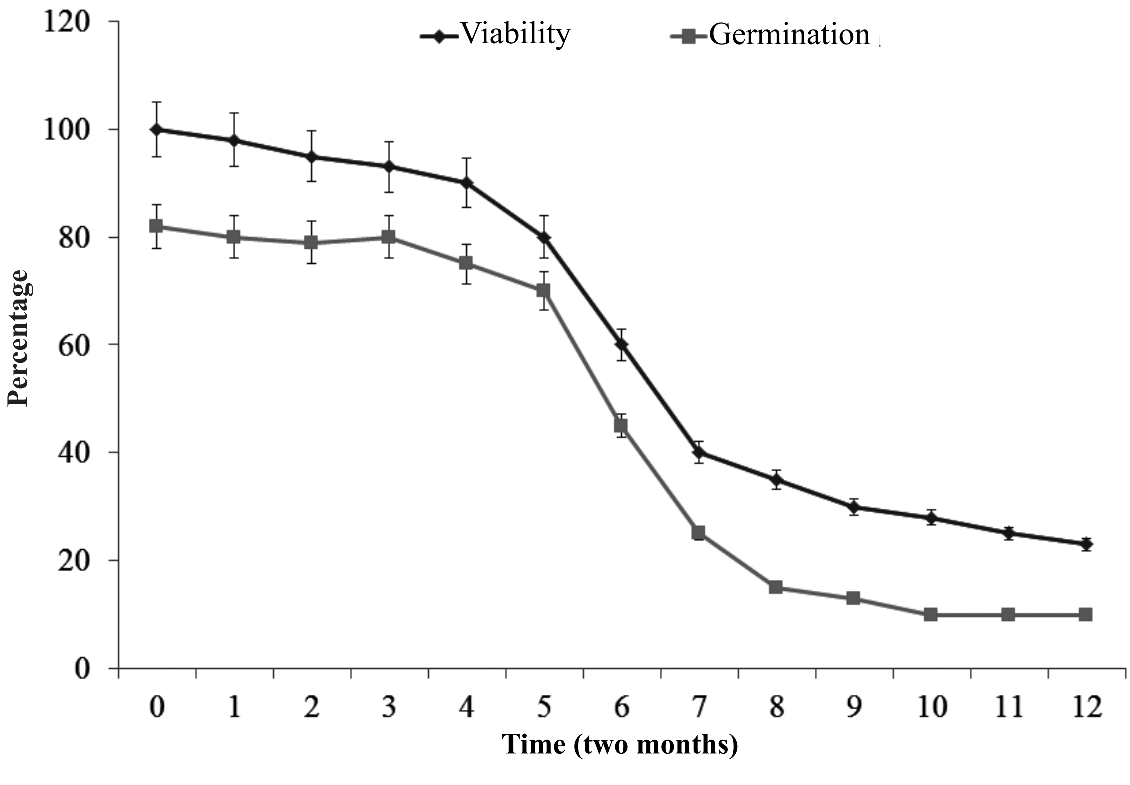
Confirming the result obtained for MGT, the MSG in T6 and T7 was the highest achieving a higher amount of germinated seeds per day, followed by T5, T4, T3 and T2, which were outstanding with respect to T1 and the Control (T0) (table).

The answers to these variables indicate that the evaluated treatments do not increase the germination percentage but decrease the time of seed germination genotype avocado evaluated, where the removal of the seed coat and the different types of apical, lateral and basal cut seed (T1, T2, T3, T4, T5, T6 and T7) (table), they can reduce the time of seed germination in 20, 24.8, 27.9, 30.5, 33, 36.8 and 39.8 days, respectively, relative to the control (T0). These results agree with other studies where it was found that the removal of the testa reduces germination time (15-17).

Meanwhile arises that if the objective of a production system avocado is to obtain seedlings rapidly and with higher rates of germination the best strategy is to use cuts in seeds (18) and as found in this work it is advisable to do cuts at the apex, lateral and basal, long as it keeps in mind that the basal cuts must be less than 5 mm to avoid damaging the embryo (Figure 1C).

Seed viability in time

The avocado seeds of evaluated genotype were characterized because its viability and germination did not decrease statistically ($p > 0.05$) to the period of 5 (six months), later it began a process of accelerated decrease ($p<0.05$) in time values in these two variables, from 90 % to 23 % for the period 12 (18 months); this variable coincided with those found for the germination percentage, which declined from 75 % to 10 % (Figure 2). In this regard it is suggested that *P. americana* seeds lose viability over time, classified as a recalcitrant seed type (19, 20).



the bars represent the confidence interval of the mean, which indicates that when there is overlap no significant differences according to Tukey test ($p>0,05$)

Figure 2. Behavior of germination and viability of seeds evaluated time genotype *P. americana* var. *guatemalensis*

For the particular case of the evaluated genotype seeds remain with high value germination and viability until eight months after storage, at which begins a process of deterioration, represented by the loss of germination capacity and decreasing the viability.

Seed mass

The first evaluation showed that the germination percentage of the seeds mass showed significant differences (p<0.05), resulting in seeds with less than 30 g mass (30-20 and <20 g) present values lower, reducing germination 60 % compared with 80 % obtained in seed size between 40 and 50 g (Figure 3A).

For the second assessment (day 60), biometric variables determined as biomass and leaf area gathered in three groups (p <0.05). In the first and the largest amount of biomass and leaf area plants from seeds were found whose mass was 40-50 g, in an intermediate place seeds 50-60 g>60 g with the lowest values whose seeds mass were less than 40 g (Figure 3 B and C). With respect to the height treatments, they were grouped into four groups with statistical differences (p<0.05). In the first and the highest values in this variable seeds whose mass 40-50 g was found, later the seeds with mass greater than 50 g (50-60 and>60 g), subsequent seed mass was between 30-40 g and fourthly with the lowest height and the seeds with mass less than 30g (Figure 3 D).

With regard to viable root masses seeds favoring this variable (p<0.05) were those between 40 and 60 g (40-50 and 50-60 g), meanwhile the other masses presented the lower values (p <0.05) decrease according decreased the seed mass (Figure 3E). Meanwhile consumption reserves in the seed for this first evaluation showed that the experimental units from exceeding 50 g masses (60-50 and> 60 g) used reserves were very low (p <0.05) resulting in consumption to less than 20 %, contrary to other treatments where stocks consumption was greater than 25 % (Figure 3F). The visual appearance of the treatments can be seen in Figure 5 A.

The destruction of the experimental to 120 days (Figure 4) units showed similar to that found at day 60, where it excelled in a pattern or trend, which was marked trend because seedlings from seeds of less than 30 g masses, development was statistically lower (p<0.05) compared to seeds of greater than 40 g mass. This trend resulted in leaf area and height are separated into three groups, the first and with highest values respect to others (p<0.05), grouped the seeds of mass 40-50 g, with intermediate values, masses exceeding 50 g and in the group with lower levels seeds with less mass than 40 g (Figure 4 AB).

For his part for biomass statistical analysis pooled the results of four different groups (p <0.05), resulting in the largest biomass present in seeds from 40-50 g mass, followed by 50-60 g in an intermediate group those mass> 60g on the lower level plantlets seed mass <40 g (Figure 4C).

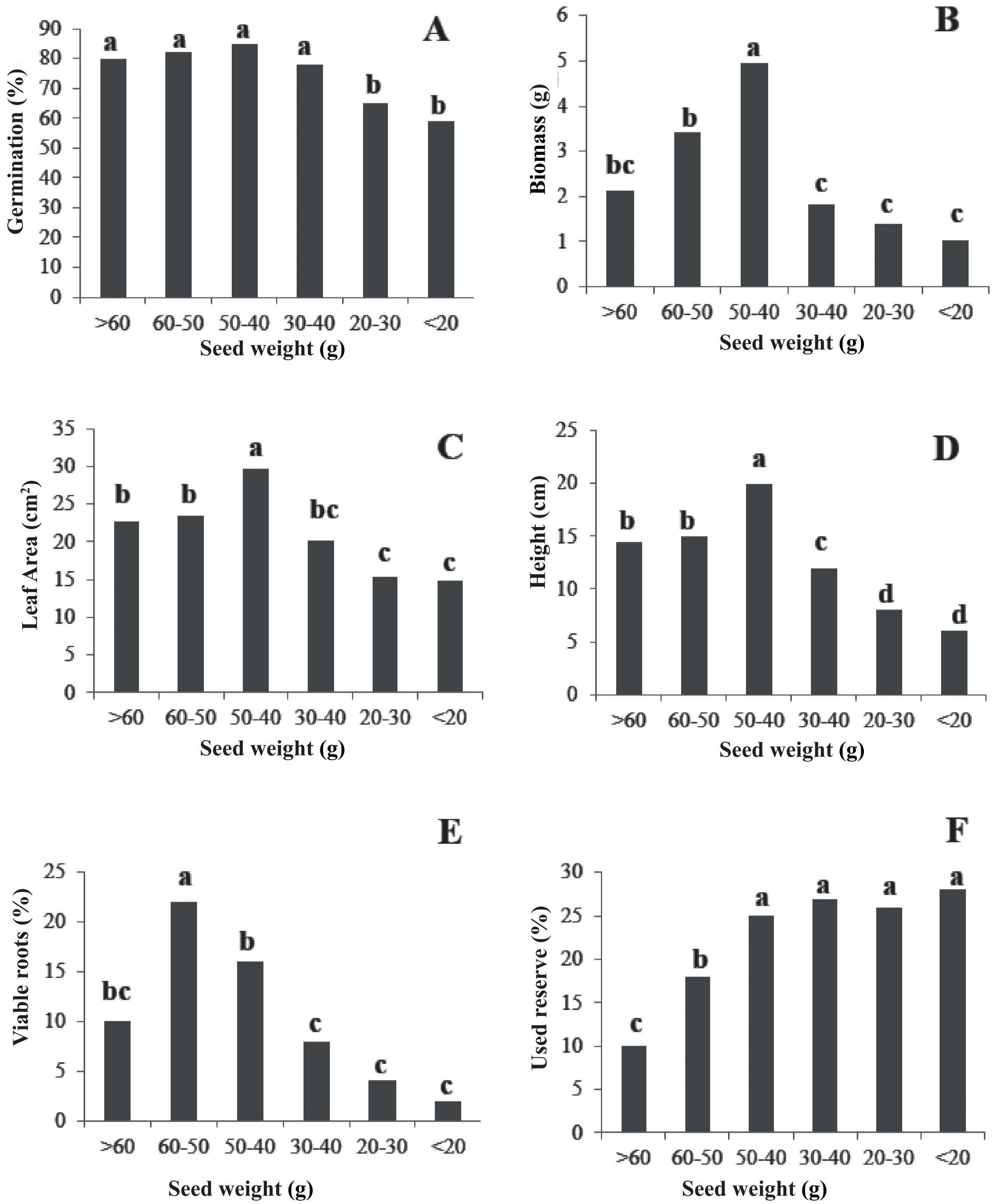
With regard to viable roots should be noted that the seeds with masses between 40-60 g, have a higher number of functional (p <0.05) (Viable), with respect to other treatments and seed mass less than 20 g are the least number of roots of this type (Figure 4 D). To this date the reserves used by the plants was over 90 % in treatments with less than 60 g mass, if not for those above 60 g, where consumption of reserves was less than 70 % (Figure 4E) . The visual appearance of the treatments can be seen in Figure 5 B.

These results suggest that the seeds of lower mass reserves are consumed quickly, contrary to the more massive, where the reserves have increased total consumption of this takes longer.

The dynamic consumption reserves by seedlings, according to mass of seeds explains the results obtained in the biometric variables, resulting seedlings from seeds lower mass, present a reduced formation of organs such as leaves and roots, which results in a reduced ability to perform metabolic processes essential for proper development. Meanwhile in the seeds of higher mass, as much reserve, resulting plantlets in their initial states present slow development, because they can get nutrients quickly from storage tissues, which results in a slower development of roots and leaves.

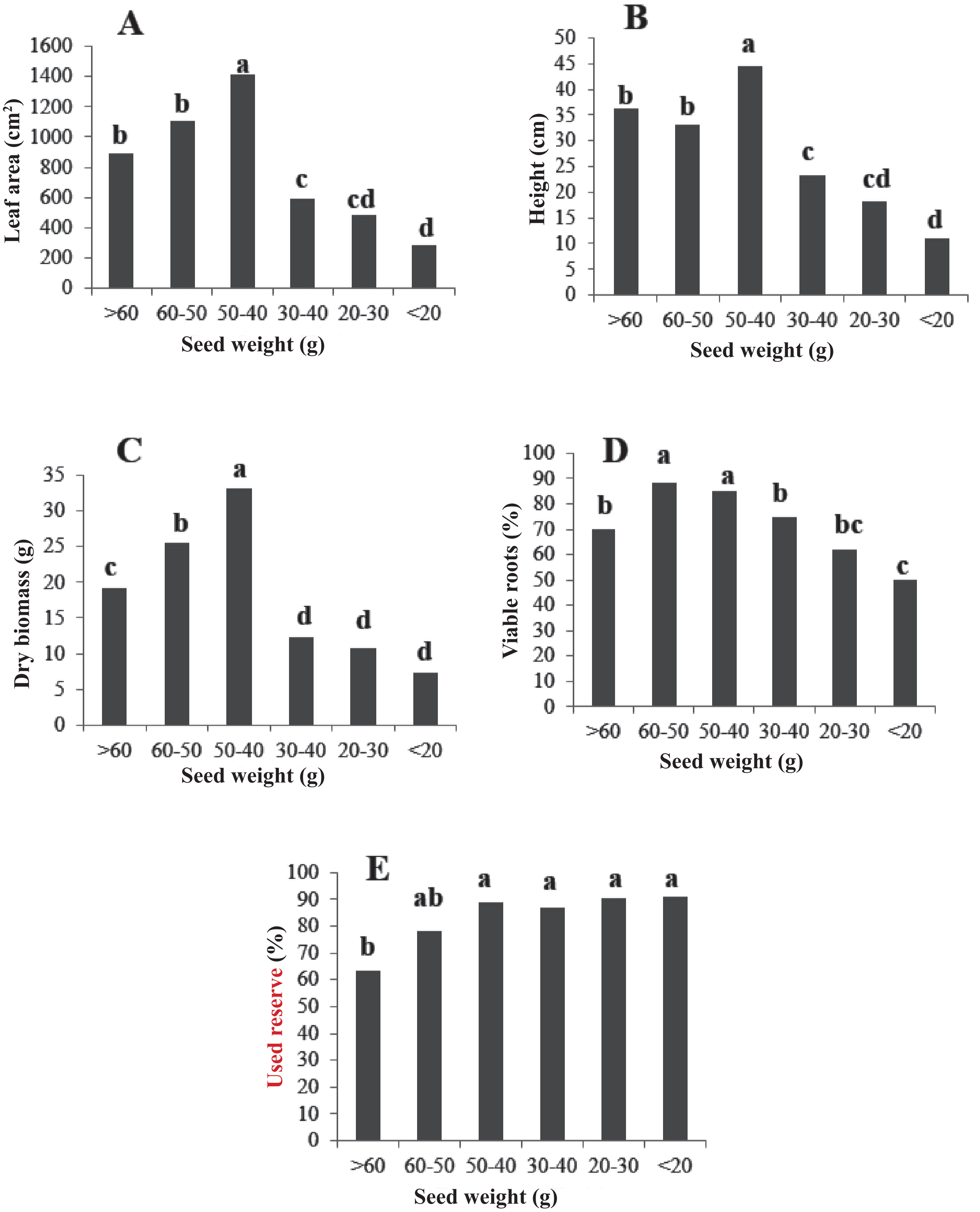
In the case of those seeds having an intermediate mass (40-50 g), reserves were used properly, which was reflected in proper development of leaves, stems and roots, which can use soil nutrients and light building plots for all metabolic processes once depleted reserves.

[D] Bárcenas, O.; Molina, E.; Huanosto, M. y Aguirre, P. "Contenido de macro y microelementos en hojas, flor y fruto de aguacate «Hass» en la región de Uruapan, Michoacán". En: V Congreso Mundial de Aguacate, 2003, pp. 365-371.



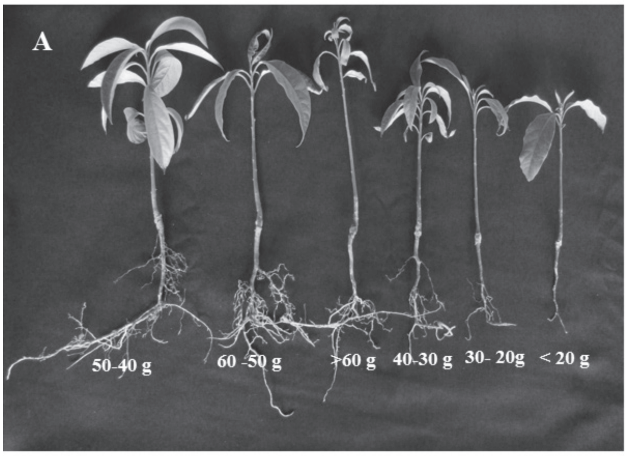
A: Evaluation conducted after germination of seedlings
B, C, D, E and F: evaluated 60 days after treatment initiation
Columns with different letters indicate that average significant differences according to Tukey test (p<0,05)

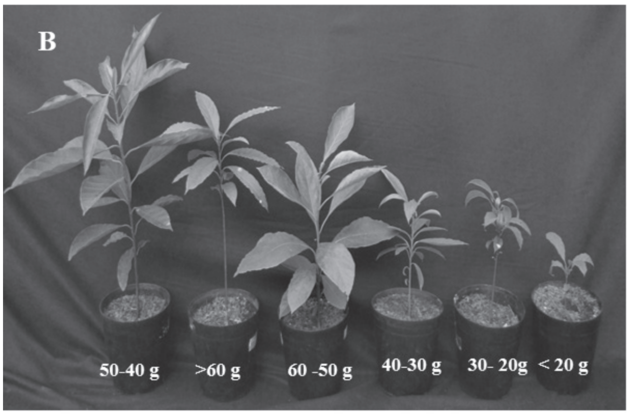
Figure 3. Biometric responses in seedlings of a genotype of *P. americana* var. *guatemalensis* from seeds of different mass to 60 days after starting treatment



Columns with different letters indicate that average significant differences according to Tukey test ($p<0,05$)

Figura 4. Biometric responses in seedlings of a genotype of *P. americana* var. *guatemalensis* from seeds of different mass to 120 days after starting treatment





A: avocado seedlings 60 days from seeds of different size
B: avocado seedlings 120 days from seeds of different size

Figure 5. Photo report of seedlings in a genotype of *P. americana* var. *guatemalensis* from seeds of different mass

In this regard it is proposed that the avocado seeds from specimens called "Creole" and possessing large seeds contain high levels of nutrients[D] (21).

Such amount of nutrients allows plants to grow well between three to five months, depending on the size, biomass and seed quality, with no other requirements than light and water, resulting in few answers on treatments that are performed in step nursery, where their development is characterized by slow growth (22). This is a problem commonly observed in *P. americana*, so it was decided to exclude this impediment, by eliminating all cotyledons and seek greater response to the different treatments evaluated in the initial stages (8).

In other plant species, it arises that the use of large seeds increases the chance of better seedlings (23-25), where some results indicate that in turn will be more robust and able to escape death processes (10).

Otherwise, it is reported that the seeds of smaller cornfield have better performance and higher germination values compared to those larger (26). Meanwhile there are some examples in which the response variables biometric seedling presented low correlation with seed characteristics such as size, mass, among others (27, 28). Despite this, few studies to explore in detail the relationship of the seed size with the use of its reserves, and the importance it may have on the seedling mass (10).

When a plant reproduces by seed, its success will depend largely on physiological and biochemical seed respects, its reaction to the environment and how quickly to use its reserves, until it is strong enough to use solar energy and nutrients ground (29).

With the results found in this study the need to determine the effect they can have variables as the mass of seeds in the response of future plantlets of various materials avocado, used as standards in commercial nurseries, arises because the large amount of materials of different origin are used. Besides, this response will be dependent on variables such as time, which is closely linked to the needs of man and the changes that this be done, because by altering some aspects as much fertilizer, microbial inoculants, environmental conditions, among others, the seeds will be more or less dependent on their reserves. However, a certain amount is necessary, given the key role in the early stages of development, when they are essential in the formation of structures necessary for a plant to express their genetic potential on favorable terms.

In this work, it was found that the germinal treatments and storage time affects germination viability and seed germination rate of a *P. americana* var. *guatemalensis* genotype. Meanwhile the use of seeds of low mass (<30 g), resulting in poor development seedlings (Figures 3, 4 and 5). In the case of higher mass seeds (> 50 g), these give rise to seedlings with slower development, resulting in that under commercial production conditions in nursery times are longer.

CONCLUSIONS

The pre-germination treatments, like storage of seeds affected the viability, germination rate, the average time of germination and germination rate of genotype *P. americana* var. *guatemalensis*., for this particular genotype optimal seed size was one with a mass of 40-50 g.

ACKNOWLEDGEMENT

With the aim of producing seedlings of avocado with a more homogeneous and shorter periods of time specific genetic identity, characterization and identification of genotype used as a pattern is recommended in addition to determine the answers to various pre-germinating treatments and evaluate the optimal size of the seed, according to the needs of the production system.

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