



Calidad de planta de *Quercus rugosa* Née en vivero *Quercus rugosa* Née seedling quality in a forest nursery

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Resumen

México reúne más especies de *Quercus* que cualquier otro país en el mundo; sin embargo, casi no hay investigaciones sobre su producción de planta en vivero, ni de indicadores de calidad. El objetivo del presente estudio fue contribuir al conocimiento de la calidad de planta de *Q. rugosa*, mediante la obtención de indicadores morfológicos, fisiológicos, prueba de crecimiento potencial de raíz y establecer el tamaño de contenedor adecuado para producirla en vivero. Bajo un diseño experimental de bloques al azar, se midieron variables morfológicas, fisiológicas (concentración nutrimental, tensión hídrica) y se hizo la prueba de crecimiento potencial de raíz. Los contenedores grandes produjeron planta con mayores ($p < 0.05$): diámetro (4.5 mm), altura (24.4 cm), peso seco total (4.3 g); así como índice de Dickson (0.6) y coeficiente de esbeltez (5.7), sin diferencias con el contenedor mediano, pero superiores al pequeño. No hubo diferencias para la relación peso seco aéreo/peso subterráneo (promedio de 1.4). Tampoco las hubo para concentración nutrimental foliar, con medias: N (1.3 %), P (0.15 %), K (1 %), Ca (0.67 %), Mg (0.45 %), Fe (88.9 ppm), Cu (12 ppm), Zn (104.7 ppm), Mn (102 ppm) y B (55 ppm). Sin diferencias entre tratamientos para tensión hídrica, aunque sí a través del tiempo: 0.58 MPa (3 d), 0.89 MPa (7 d) y 2.74 MPa (11 d). La planta en contenedores grandes emitió más raíces nuevas (media de 65.7) y es la más apropiada para reforestación. Los indicadores de calidad obtenidos pueden ser útiles para la especie estudiada y para otras del género.

Palabras clave: Contenedores, indicadores de calidad de planta, producción de planta, *Quercus rugosa* Née, raíz, viveros forestales.

Abstract

Mexico is the world's richest country in *Quercus* species, but there is very scarce research on how to produce it in forest nurseries as well as in seedling quality indicators. The objective was to establish seedling morphological, physiological and root growth potential quality indicators, and to determine the best container size to produce the species in the nursery in a randomized blocks experimental design. Were measured morphological, physiological (nutrient concentration, hydric stress) and was performed a root growth potential test. The big containers produced plant with the highest caliper (4.5 mm), height (24.4 cm), total dry weight (4.3 g); as well as Dickson index (0.6) and slenderness coefficient (5.7), without differences with the medium size container but higher than the small one. No differences were found for the shoot/root ratio (average of 1.4). Neither for the foliar nutrient concentration with the following averages: N (1.3 %), P (0.15 %), K (1 %), Ca (0.67 %), Mg (0.45 %), Fe (88.9 ppm), Cu (12 ppm), Zn (104.7 ppm), Mn (102 ppm) and B (55 ppm). There were no differences among treatments for hydric stress, but there were towards time: 0.58 MPa (3 d), 0.89 MPa (7 d) and 2.74 MPa (11 d). The plant from bigger containers emitted more new roots (average of 65.7) and is better for reforestation. The seedling quality indicators obtained in this study may be useful for the studied species and for others of the genus.

Key words: Containers, seedling quality indicators, seedling production, *Quercus rugosa* Née, root, tree-forest nurseries.

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Introduction

Mexico is a mega-biodiverse country from its geographical location and physiographic and climatic variation. As part of this biodiversity, it has 170 species of oaks, which defines it as the country with the greatest richness of the *Quercus* genus on the planet, followed by China with 150 species, while worldwide there are 450 of them (Zavala, 2007). In the whole nation, oaks live in temperate-cold, semi-arid and tropical zones and are present in all states, except for *Yucatán*. The genus grows in areas with average annual rainfall between 350 and 2 000 mm, from zero to nine months of drought, and altitudes from 0 to 3 500 m. It forms pure stands (of species of the genus) or mixed, mainly with pines (Zavala, 1995, 2003; Rzedowski, 2006). There are 9 518 561 ha with broadleaf forests in Mexico, mainly *Quercus*, and 14 499 659 000 ha of conifers and broadleaves, most of them *Pinus-Quercus* (UACH-Semarnap, 1999).

These forests provide environmental services, such as carbon and water capture, erosion prevention and enrichment of the soil with organic matter, and host or are a matrix for a great variety of plant and wildlife species, such as squirrels, birds, wasps, bees, flies, beetles, orchids, lichens, bromeliads, ferns and climbers (Arizaga *et al.*, 2009).

The rural communities use oaks wood to make charcoal, and firewood as well as raw material to build houses, furniture, agricultural implements, handicrafts, among the most valuable. Acorns of several species are fed to livestock. At the industrial level, with oak wood handles for tools, furniture and staves are made, among other products are made. The grasslands associated with various oak groves and pine-oak forests are of livestock importance (Arizaga *et al.*, 2009).

Quercus rugosa Née is one of the most widely distributed oaks in the country. It is found in 23 states, between 1 100 and 3 000 m asl. It is a tree 8 to 30 m high, with a diameter to the breast height of 10 to 80 cm, even larger, a wide rounded crown and slow growth. It is evergreen or shortdeciduous, flowers in March-June and bears

fruit in October-February. It is used for coal, firewood, lines, piles and sleepers; in some areas its fruit is used as a substitute for coffee (Vázquez *et al.*, 1999; Arizaga *et al.*, 2009; Conabio, s/f).

In order to be successful in establishing plantations, whether for reforestation, restoration or commercial use, the probability of survival in the field increases by handling quality plants. The quality plant is one that has certain morphological and physiological properties that allow it to establish, grow and develop vigorously in the planting site (acclimatize) (Rodríguez, 2008; Prieto *et al.*, 2009).

The nursery production of quality plants in Mexico is becoming very important to improve survival in the field and reduce replacement costs. However, this information is mainly concentrated on pine species due to its forest and economic importance (Escobar-Alonso and Rodríguez, 2019). Although the *Quercus* genus is as ecologically relevant as *Pinus*, it does not have the same economic importance, which is why it has been studied in a smaller scale.

Based on the lack of the aforementioned information, the objectives of this study consisted on providing knowledge of the quality of the *Q. rugosa* plant, by obtaining morphological and physiological indicators, testing potential root growth and establishing the container size more convenient to produce the species in the nursery.



Materials and Methods

Plant production

The plant production was made in the forest nursery of the Forest Sciences Division (Dicifo) of the *Universidad Autónoma Chapingo* (Chapingo Autonomous University) (UACH), located in the State of Mexico, at 19°29'46 "N and 98°52'14" W, at 2 250 masl. According to the Köppen climatic classification modified by García (1973), the climate in the nursery area corresponds to a subhumid temperate C (W_o)W_b(i')g, with a rain regime in summer, little thermal variation, average annual temperature of 17.1 °C, average maximum of 23.5 °C and average minimum of 6.3 °C, as well as an average annual rainfall of 686 mm.

The substrate used consisted of a mixture of vermiculite (0.114 m³ = 36 %), agrolite (0.05 m³ = 16 %) and peat moss (0.15 m³ = 48 %). Three different container capacities were tested: large (210 cm³, black, rigid, circular cross section, five drainage holes, 14 cm high and 5 cm top diameter, 54 cavities per tray); medium (135 cm³, black, polypropylene, semi-rigid, octagonal cross section, one bottom drainage hole, 12 cm high and 5 cm top diameter, 50 cavities per tray); and small (93 cm³ -V93-, black color, rigid, circular cross section, 9 cm in high and 4 cm in top diameter, one drainage hole, 40 cavities per tray). The equivalent of 4 kg m⁻³ of Osmocote™ granular fertilizer (formulation 15-9-12 N, P₂O₅, K₂O, 5-6 months of release) was added.

Sowing was carried out in October 2010, with a total of 2 694 seeds in 57 trays, to produce enough plant for all tests. In each container a seed was placed 1 cm deep. The apex of the nut, where the radicle emerges, was centered in the pot, to favor the symmetrical development of the root. The trays were placed in a tunnel-type greenhouse, where mycorrhizae were applied: 50 g of mycorrhizal inoculum of *Pisolithus tinctorius* (Pers.) Coker *et* Couch (Ecto-rhyza TM, Plant Health Care de Mexico). At the same time, *Trichoderma harzianum* Rifal was added (strain T-22, Plant Health Care *de México*) as antagonistic fungus of phytopathogens.

The cultivation stages, defined primarily by fertilization and irrigation, as described in the following paragraphs, had the following duration: establishment (October and November 2010); growth I (November and December 2011); hardening I (January and February 2011); growth II (March to July); and hardening II (August and September 2011). The cultivation was done in a greenhouse until the first two months of growth stage I, during which a shade mesh was used (green, 70 %).

Fertilization was divided into three phases, according to the development of seedlings and saplings: the establishment phase (goals of N, P and K: 50, 100 and 100 ppm), the growth phase (goals: 150, 60 and 150 ppm) and that of hardening (goals 50, 60 and 150 ppm). In each stage, the fertilizers were used, respectively: starter (Peters™ 8-45-14 of N, P₂O₅ and K₂O), growth (Peters™ 20-20-20) and finisher (Peters™ 4-25-35). To achieve the goals (N, P and K per stage), 0.62, 0.75 and 0.52 g L⁻¹ of the starter fertilizers, for growth and finisher already referred, were applied.

Watering was made according to the cultivation stage: light, maximum at field capacity and frequent (establishment); heavy, maintaining field capacity, every third day (growth); and every 2-3 days at field capacity (hardening).

As the presence of mosquitoes and aphids that caused leaf curl and yellowing was noted, dimethoate pesticide (1 mL L⁻¹ water) was applied to control them. Furthermore, when *Oidium* sp. was detected, it was initially treated with the fungicide Benomyl (0.9 g L⁻¹ water), but as the expected results were not accomplished, it was finally treated with Rally 40W (Myclabutanil) (0.3 g L⁻¹ water). These effects were controlled at first, and thus, they did not influence plant quality.



Morphological indicators

An experimental design of complete random blocks was used, with four replications (trays), for each of the three treatments (210, 135 and 93 cm³ containers) and 144 plants per block. The variables measured for the morphological analyzes were: height, which was measured from the root collar to the apical bud (with a 30 cm rule); diameter, at the height of the root collar (with a 0.1 mm Truper digital vernier); number of leaves and shoot/root ratio (Figure 1). For the latter, when the plant was 11 months old, the dry weight of the shoot and the root was obtained, by dividing each plant into stem, leaves, main root, secondary roots and placing them in brown paper bags inside an H41 Ríos Rocha™ oven at 70 °C until the dry weight of these components is reached; weighings were performed with a N14120 (0.01 g) Ohaus digital scale every third day, until constant weights were reached. The Dickson quality index [ICD = total dry weight (g)/(shoot (g)/root (g) ratio + slenderness index (cm/mm))] and the slenderness index (height (cm)/diameter (mm)) (Landis *et al.*, 2010) and the phytosanitary status of the plants was observed. This phase of the research was developed in the Forest Seed Laboratory of Dicifo, UACH.





Figure 1. Sample of containers and plant produced. From left to right: large, medium and small containers. These samples were analyzed in their morphological characteristics in the laboratory.

Physiological indicators

To determine the nutritional concentration, 12 11-month-old plants (three for each treatment) were randomly selected and taken to the Central University Laboratory of the UACH to carry out such analysis: for all the nutrients, digested with a diacid mixture was used. The determinations were made by vapor entrainment (N), molybdo-vanadate reduction focolorimetry (P), flame emission spectrophotometry (K), atomic absorption spectrophotometry (Ca, Mg, Fe, Cu, Zn and Mn) and by photocolorimetry with azomethine-H (B).

In the water stress test (started on August 31st, 2011), an initial irrigation was applied and it was suspended until the end of 3, 8 and 11 days after such irrigation. The dates of the measurements were: September 3rd, 8th and 11th, 2011. The readings were made in branches, before dawn (before transpiration). 12 plants were used for each treatment (container size); in each record, three measurements were made per treatment. The water stress measurements were made with a 1000 PMS Instrument Scholander chamber (pressure pump).

Root growth potential

For this test, a randomized block design was used, with five replications and each block with three 2 L pots (one per container size treatment) and five plants per pot. One more was used to determine the appropriate time for the registration of new roots (16 pots and 80 plants in total). They were filled with a substrate in equal parts of peat moss, vermiculite and fine sand (1: 1: 1), under greenhouse conditions, with moderate irrigation, for a month (October 2011). After that time, new roots (white and succulent) greater than 1 cm in length were counted.

Statistical analysis

For the statistical analysis was utilized the Analysis of Variance (PROC ANOVA) procedure of the SAS v. 9.0 (SAS Institute, 2002). In addition, the least significant difference test was used to determine the differences between treatments. In all cases, the statistical model was:

$$y_{ijk} = \mu + a_i + \beta_j + E_{ijk}$$

Where:

y_{ij} = Response variable of the i^{th} block, in the j^{th} treatment

μ = General mean of the experiment

a_i = Effect of the i^{th} block

β_j = Effect of the j^{th} treatment

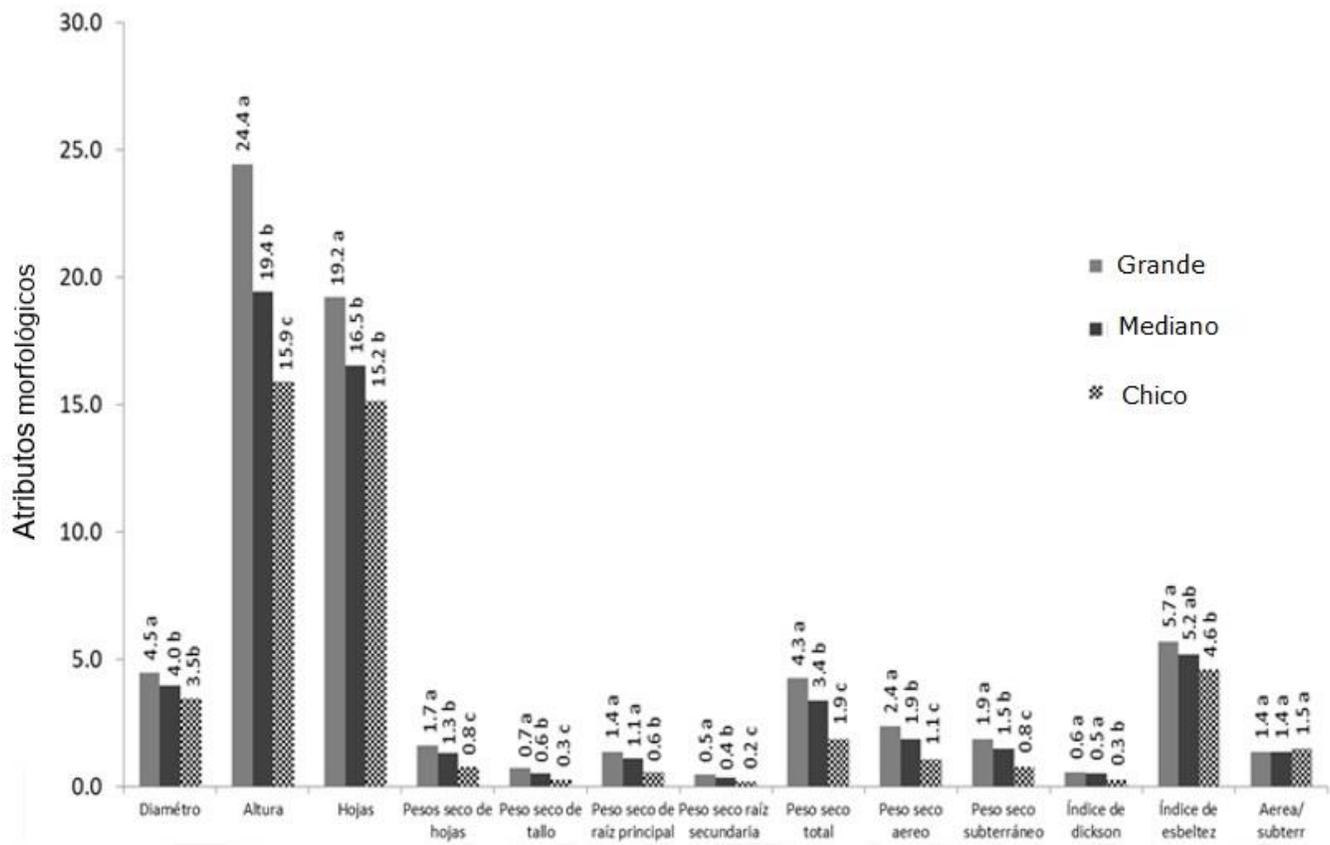
E_{ijk} = Experimental error

Results and Discussion

Morphological analysis

For the diameter variable, significant differences were obtained between the treatments ($P = 0.0007$), since the large container had an average of 4.5 mm, while the medium and small containers did not present statistical differences between them (4.0 and 3.5 mm, respectively) (Figure 2). The diameter of the root collar is a good predictor of the growth and future survival of a plantation (Ruano, 2003). A larger diameter is related to a more vigorous root, greater potential to store carbohydrates, greater stiffness to tolerate moisture limitations and mechanical damage, and more buds for regrowth if necessary (Landis *et al.*, 2010).





Atributos morfológicos = Morphological attributes; *Diámetro* = Diameter; *Altura* = Height; *Hojas* = Leaves; *Peso seco de raíz principal* = Main root dry weight; *Peso seco raíz secundaria* = Secondary root dry weight; *Peso seco total* = Total dry weight; *Peso seco aéreo* = Shoot dry weight; *Peso seco subterráneo* = Root dry weight; *Índice de Dickson* = Dickson's index; *Índice de esbeltez* = Slenderness index.

Figure 2. Morphological attributes, according to the size of the container. The units are as follows: diameter (mm), height (cm), dry weights (g). The indexes do not have units. Different letters in the same thirds of bars indicate significant differences between treatments.

This characteristic defines the robustness of the stem and is associated with the vigor and success of the plantation, since, while the diameter is > 5 mm, the plants are more resistant to bending and better tolerate damage by pests and harmful fauna,

which varies with the species (Prieto *et al.*, 2003, 2009). All the diameters obtained were within the range of 3.5 to 4.8 mm referred to quality plants of *Q. ilex* L. in Spain (del Campo *et al.*, 2010).

Significant differences were recorded between treatments for height ($P < 0.0001$). The height of the plant produced in a large container was greater (24.4 cm) than that of the medium (19.4 cm) and small (15.9 cm) containers (Figure 2). According to the results obtained by Cortina *et al.* (1997), *Quercus rotundifolia* Lam. in Spain it showed a significant relationship between height and diameter with survival, the opposite of which was observed for *Quercus coccifera* L. in the same study.

In studies on the quality of the plant produced in the forest nurseries of Jalisco (Rueda *et al.*, 2012), intervals were established for the morphological attributes evaluated in native broadleaves (but not *Quercus*) and exotic, both temperate-cold climates and tropical climate. Those of height were: low (< 12.0 cm), medium (12.0-14.9 cm), and high (≥ 15.0 cm). For their part, Sáenz *et al.* (2010) indicated that the broadleaves must reach a height of 20 to 35 cm to be considered of quality. The heights of the seedlings in the present study are equal to or greater than 12-17 cm for *Q. ilex* in Spain (del Campo *et al.*, 2010).

Quercus rubra plants of greater height and diameter at the time of planting had greater survival in the United States the first years, although an important effect of the compared families was also observed (Pinchot *et al.*, 2018). A similar trend was observed in *Q. rugosa* produced in plastic bags and planted in conditions of limited humidity in Sierra de Guadalupe, Edo. de Méx. There the large plant (height, 16-24 cm; diameter, 2-4 mm), on NE slopes, planted with a microsite (next to and NE rock), had a 63 % survival, while the small one (height, 8-15 cm; diameter, < 2 mm) and without a microsite, it had zero survival (Ramírez and Rodríguez, 2004).

The number of leaves showed significant differences ($P = 0.0003$), between the large container and the medium and small containers (Figure 2), without differences between the latter two. A similar trend, but with all container sizes differing from each other, was observed

for foliage dry weight ($P < 0.0001$). As long as there is a greater biomass in the leaves, there will be a greater capacity in the plant to capture light and CO_2 , which results in a higher photosynthesis, growth rate, or, in a greater allocation of biomass to the roots, which leads to capture more water and mineral nutrients from the soil, but at the expense of less growth in the aerial part (Villar *et al.*, 2004).

Values were higher in the largest containers, with differences between the three container sizes, for dry weight of stem, secondary roots, total dry weight, shoot and root dry weight ($P < 0.0001$). There was no difference between the large and medium containers, but it was with the smaller one, in dry weight of the main root and Dickson's quality index ($P < 0.0001$). Sáenz *et al.* (2010) and Rueda *et al.* (2012), agreed that higher ICD values corresponded with the best results in plantation, that is, quality plants, so that the large and medium containers are congruent with the high quality ranges.

For the slenderness index, there were only differences between the large and small containers ($P = 0.0541$), while for the shoot / root ratio, there were no differences ($P = 0.5568$) between the container sizes (Figure 2). This indicates that, although the plant shows differences in total dry weight or of the aerial or underground parts, proportion between both was kept the same between the different containers.

The shoot/root ratio with low values favors survival in sites with humidity limitations (Cano and Cetina, 2004). Rueda *et al.* (2012) reported that the ranges of this variable, established for forest plants in general, classify them as low ≥ 2.5 , medium 2.4-2.0 and high < 2.0 ; the latter of which agrees with the values recorded in the three container sizes (Figure 2).

In general, it is recommended that the slenderness index be less than 6 (Rodríguez, 2008). If it is excessively tall (> 6), it is indicative of slender plants, with stem elongation disproportionate to the growth in diameter (Thompson, 1985) (Figure 2).

Physiological tests

For the elements considered in the nutritional analysis, there were no significant differences in their concentration in the different container sizes. Apparently there are no standard levels of foliar nutrient concentration for national oaks, although the concentrations found in the present work for N, P and K, remain within or above the intervals established by del Campo *et al.* (2010) for an oak from Spain, *Q. ilex* (1, 0.09 and 0.37 %, respectively). For his part, McCreary (2009) recommended 100 ppm N with 20-20-20 fertilizer for California oaks, similar to the fertilization handled in this work.

Landis *et al.* (1989) defined foliar nutrient concentration standards for conifers in nurseries, information that can serve as a general guide. The proportions are as follows: N (1.4-2.2 %), P (0.2-0.4 %), K (0.4-1.5 %), Ca (0.2-0.4 %), Mg (0.1-0.3 %), S (0.2-0.3), Fe (60-200 ppm), Mn (100-250 ppm), Zn (30-150 ppm), Cu (4-20 ppm), Mo (0.25-5.0 ppm) and B (20-100 ppm). Other authors present nutrient concentration ranges for forest species in nursery in general, considering the following adequate (high quality): N > 1.3 %; P > 0.2 %; and K > 0.7 %. Medium quality: N 1.0 - 1.2 %; P 0.1-0.2 %; K: 0.5 -0.6 %. Low quality: N <1.0; P <0.1 %; and K: <0.5 % (Santiago *et al.*, 2007; Conafor, 2009; Sáenz, *et al.*, 2010; Rueda *et al.*, 2012). Taking into account the given ranges, almost all the concentrations of the elements analyzed for *Q. rugosa* (Table 1) are within the established intervals for the plants of high to medium quality. Only P was slightly lower, and Ca and Mg slightly higher at such concentrations. However, no visual symptoms of P deficiency were noted in plants; Bennett (1993) and Alcántar *et al.* (2013) point out that high levels of Ca and Mg do not generate toxicity in crops.



Table 1. Results of the nutrient analysis.

Nutrient	Container		
	Big	Medium	Small
N (%)	1.3 a	1.2 a	1.3 a
P (%)	0.15 a	0.14 a	0.16 a
K (%)	0.97 a	1.03 a	1.04 a
Ca (%)	0.69 a	0.70 a	0.61 a
Mg (%)	0.47 a	0.45 a	0.45 a
Fe (ppm)	95.7 a	86.6 a	84.3 a
Cu (ppm)	10.76 a	11.72 a	13.60 a
Zn (ppm)	100.1 a	105.1 a	108.8 a
Mn (ppm)	101.3 a	110.3 a	95.7 a
B (ppm)	52.3 a	58.7 a	53.8 a

Columns with the same letter do not have significant differences between them ($P \leq 0.05$).

For water stress, the analysis indicates significant differences between the days on which the measurements were made ($P = 0.0001$), while there were no differences between the containers ($P = 0.6355$). The means per day were: 0.58 MPa (3 d), 0.89 MPa (7 d) and 2.74 MPa (11 d), which establishes that the water stress of the oak increases as the time without irrigation increases.

Landis *et al.* (1989) report that values close to 0 MPa indicate low water stress, 1.0 MPa indicates moderate stress and 2.0 MPa, high stress. The above coincides with the results obtained in the present work, since after 11 days without watering the plants, their water tension was high. Species have different sensitivity to water stress, so it is advisable to water in the early morning, when the water potential decreases below -0.5 MPa; with values from -1.0 to -1.5 MPa, growth is restricted and preconditioning is variable; if the water potential is between -1.5 and -2.5 MPa, stress is severe, and there is a risk that plants suffer damage and die (Landis *et al.*, 1989).

Bonfil (1998) and Bonfil (2006) pointed out that, in disturbed scrubs, the initial survival of *Q. rugosa* is closely linked to water availability, as well as to herbivory.

Root growth potential test

Significant differences between treatments were verified ($P = 0.0048$). The mean of the large container was 65.7 new roots, 54.4 in the medium container, and 52 in the small one. However, there was no difference between the medium and the small container. The results obtained were analyzed with the root growth index (RGI) scale developed by Tanaka and contributors (Landis *et al.*, 2010), in which most of the plants had between 31 -100 new roots (corresponding to a Tanaka index equal to 5), so there were no differences. The higher this value, the better development of the trees in the plantation is expected.

The root growth potential test is considered one of the most reliable for plant quality when assessing the response potential of a commercial plantation (Van den Driessche, 1984). The importance of a fibrous root system in *Q. rubra* L. produced in trays was evident in Canada, as they obtained 100 % survival in the first year (Wilson *et al.*, 2007). The degree of pre-adaptation of the plants to the site conditions will be the factor with the greatest influence on the behavior of the plants during the initial development period after transplantation (Ortega *et al.*, 2006) and a good initial conformation of the plant roots is a good indicator of such ability.



Conclusions

The large container produces the largest plant with the highest potential root growth, as it provides more room for growth. It is considered that this type of seedling exhibits better survival in the field, even in places with humidity limitations. The morphological indicators and the nutrient concentrations established for *Q. rugosa* in this work can serve as a reference for the production of this and other species of oaks in nurseries. It is necessary to carry out more research on morphological and physiological indicators and plant quality tests as well as the effect of seed size for different *Quercus* species to have information that helps improve survival in the field of reforestations with species of this genus.

Conflict of interest

The authors declare no conflict of interest.

Contribution by author

Francisca De Jesús Albino and Rosalina Ignacio Hernández: field and laboratory work, data analysis and processing, information search and writing of the manuscript; Dante Arturo Rodríguez Trejo: design of the experiment, field and laboratory work, statistical analysis, supervision of the investigation, writing and editing of the final paper; Leopoldo Mohedano Caballero: support in the design of the research, supervised the research and editing of the final writing.

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