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Review article

Estado del arte en la investigación sobre calidad de planta del género *Pinus* en México

State of the art of the research on seedling quality of the genus *Pinus* in Mexico

Sebastián Escobar-Alonso* y Dante Arturo Rodríguez Trejo

Resumen

Las investigaciones sobre calidad de planta son necesarias para orientar la producción en vivero y contribuir a mejorar la supervivencia en campo de los individuos utilizados en programas de reforestación. Para indagar acerca del uso de variables morfológicas, fisiológicas y pruebas usadas en la determinación de la calidad de planta de especies nativas del género *Pinus* en México, producidas en vivero, se realizó una búsqueda de artículos científicos sobre el tema, publicados del año 2000 hasta mediados del 2018 en diferentes bases de datos. Se encontraron 61 artículos científicos (77 estudios). El diámetro y la altura resultaron ser los atributos morfológicos más utilizados, seguidos de la relación peso seco parte aérea entre peso seco de raíces. Respecto a los fisiológicos sobresalieron la concentración foliar de nutrientes y el potencial hídrico. En menos de un tercio de las investigaciones se llevó a cabo la comprobación de supervivencia en campo; por lo que, es importante aumentar el número de estudios respaldados con el uso de esta prueba, para dar mayor robustez y aplicabilidad a los resultados. Solo se han tratado, formalmente, 17 de los 57 taxones de pinos presentes en México. Dada la relevancia ecológica y económica del género, es importante continuar la investigación con los taxa faltantes, y profundizar en los ya considerados sobre temas como tipo de especies de hongos micorrícicos, su abundancia e intensidad de micorrización en las plantas, entre otros.

Palabras clave: Calidad de planta, estado del arte, índices de calidad, pinos, reforestación, viveros forestales.

Abstract

Research on seedling quality is necessary to guide nursery production and to contribute to the improvement of survival in the field in reforestation programs. In order to investigate the use of morphological and physiological variables as well as the tests used for determining the quality of seedlings of nursery-grown Mexican *Pinus* species, a review of the subject was carried out in scientific articles published between the years 2000 and mid-2018 in different databases. 61 scientific articles (77 studies) were found. Diameter and height were the most used morphological attributes, followed by the shoot:root ratio. The most used physiological traits were the leaf concentration of nutrients and the water potential. Less than one third of the research includes the performance of field survival tests; more studies should be supported using this test in order to render the results more robust and applicable. Only 17 out of the 57 pine taxa in Mexico have been formally researched. Given the ecological and economic relevance of the genus, it is essential to continue researching the other species and to delve further into such topics as the analysis of micorrhyzal species and the level of micorrhyzation in plants.

Keywords: Seedling quality, state of the art, quality indexes, pines, reforestation, forest nurseries.

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[1] Universidad Autónoma Chapingo, México
*Autor por correspondencia; correo-e: sebastian9477@gmail.com

Introduction

Pine forests have a great ecological and economic importance in Mexico. They are often the main component of vegetation, influencing ecosystem processes, such as the hydrological and biogeochemical cycles; they are the habitat and source of food of wildlife, and they provide timber, firewood, pulp, resins, edible seeds and other non-timber forest products (Sánchez, 2008).

Mexico is a center of diversification of the genus *Pinus*, and in its territory includes the largest number of their taxa; this is caused, to a great extent, by orographic and climatic processes occurred in the geologic past (Challenger and Soberón, 2008). Gernandt and Pérez (2014) point out that the Mexican Republic hosts 57 of the approximately 120 taxa that make up the genus. According to the International Union for the Conservation of Nature (UICN, 2001), 35 species exhibited a status of least concern; seven are vulnerable; eight are close to being threatened; six are endangered, and one has an unknown status.

The Mexican nation is part of a global phenomenon in which the pine forests are becoming fragmented and modified, mainly due to the introduction of non-native species, illegal logging, and the expansion of agricultural land and of forest fires (disturbed fire regimes) (Chapa *et al*., 2008; Ortíz *et al*., 2008; Bekele, 2011; Otavo and Echeverría, 2017). This renders the implementation of programs crucial to the conservation and propagation of the species at risk, as well as of those that are not at risk, through strategies for achieving a sustainable supply of germplasm or planting material (Broadhurst *et al*., 2015). This scenario generates the need for the production of plants in nurseries.

The establishment in the field of the produced individuals is limited by man-made factors, such as grazing, and by natural phenomena (frost and drought). In the case of the latter, they largely affect plant quality (Bautista *et al*., 2005; Robles *et al*., 2017; Prieto *et al*., 2018), which can be defined as the morpho-physiological characteristics of the plant that allow its survival, growth and development at the planting site (Duryea, 1985). Those characteristics that constitute an evidence of quality depend on genetics, but also on the propagation technology (Rodríguez, 2008; Prieto *et al*., 2009). The present work uses the term “seedling quality”; however, other terms have been utilized, as shown below.

The evolution of the concept of seedling quality was analyzed by Grossnickle and MacDonald (2018), who point out that the term has changed from “desirable seedlings”, *i.e.* seedlings selected based on their vigor and growth capacity, to “target seedlings”, *i.e.* seedlings with morphological and physiological characteristics that can be linked quantitatively to survival at the planting site (Mexal and Landis, 1990); all the way to the operational implementation of the latter concept and its expansion to “target plant” (including herbaceous shrub and tree species) (Landis, 2011) and its application in the relationship between nursery growers and reforesters, with the purpose of achieving specific reforestation goals (Dumroese *et al*., 2016).

Seedling quality is determined based on a series of morphological and physiological parameters, as well as on tests that help predict if individuals to be planted will survive. The most commonly used morphological attributes are the diameter (D), height (H), total dry weight (TDW), total fresh weight (TFW), dry weight of the aerial part (ADW), and dry weight of the roots (RDW). Indexes interrelating the above variables have been proposed, including the slenderness index (SI), the shoot:root ratio, Dickson’s index (DI) (Dickson *et al*., 1960), and the lignification index (LI). Commonly utilized physiological attributes are the water potential, the concentration of nutrients and non-structural carbohydrates (NSC), the net photosynthesis and the mitosis rate, among others. Also, there is evidence of behavior that includes the potential root growth (PRG) and resistance to cold and drought, among others (Duryea, 1985). There are quality intervals for the morphological attributes and certain physiological attributes of several Mexican conifer species, which allow classifying the quality of (cespitous and non-cespitous) pines and broad-leaved species, proposed by Sáenz *et al*. (2010) and modified by Rueda *et al*. (2014).

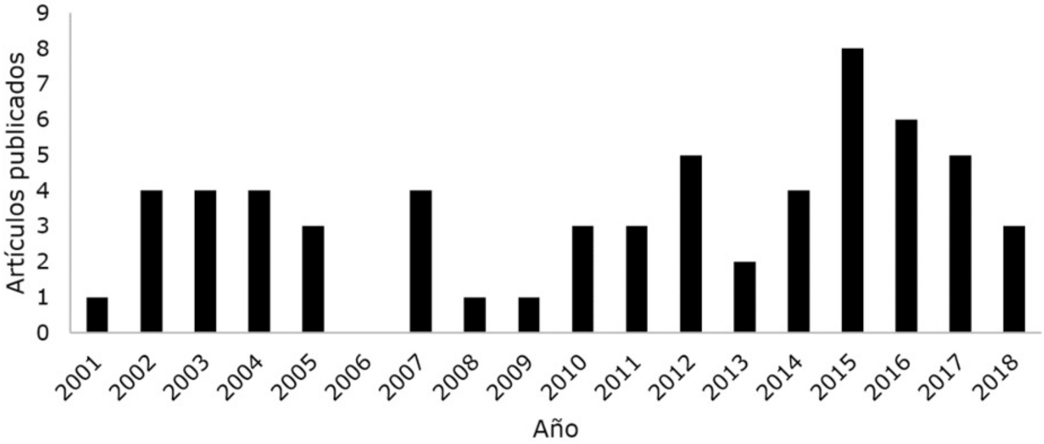
Numerous parameters have been included in the research on Mexican pines. However, there are no analyses of the frequency of use, nor of the success of each one of them or of the studied species. Based on the above, the objective of this literature review is to inquire about the use of morphological and physiological variables and of performance tests to determine the quality of nursery-grown native species of the genus *Pinus* in Mexico, with the purpose of carrying out a diagnosis and help perceive research opportunities on that topic.

Methodology

Literature search was conducted in the databases: *ScienceDirect*, *Scopus*, *STOR*, SciELO, *Springer*, Redalyc and *Google Scholar*. Literature was sought both in English and in Spanish, and the search was limited to scientific journal articles published between the years 2000 and mid-2018. No citations of previous years were included because they are practically non-existent. The words used were concentrated in title and keywords related to the nursery production of plants and in the plant quality of Mexican pine species; for example: quality, seedling quality, *Pinus*, Mexico, morphological parameters, physiological parameters, and survival. Given that some articles include more than one species, the research on each particular species was termed "study".

Research and studied species

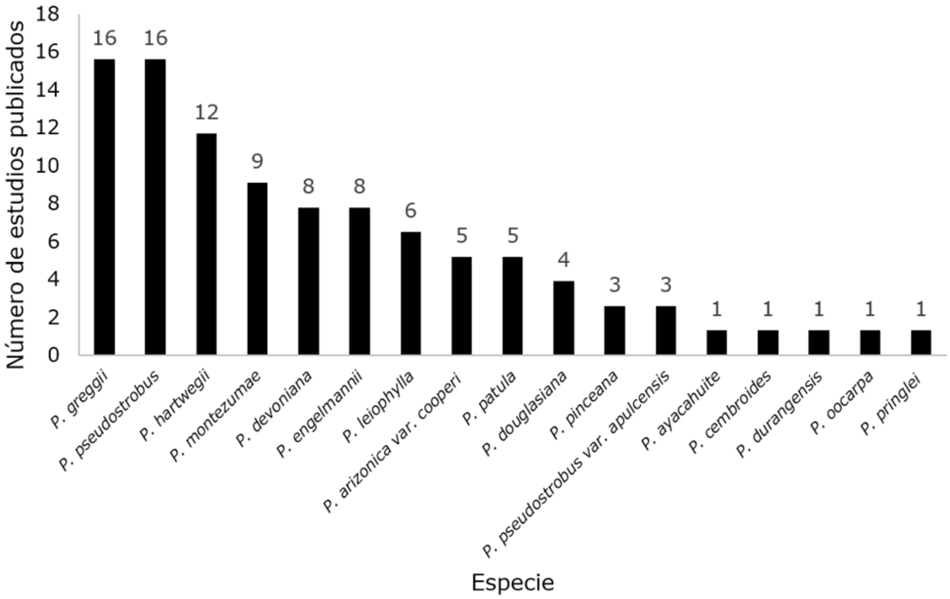
For the period under consideration, 61 articles were found, corresponding to 77 studies on the subject and the genus of interest, with an average of 4.3 per year. The variability was 0 (2006) to 8 (2015). Although there is no direct annual trend in the increase of publications, notably two thirds were published in the last 10 years (Figure 1).



Año = Year; *Artículos publicados* = Published articles

Figure 1. Published articles in scientific journals about plant quality of Mexican pines.

The hitherto published studies cover only 17 (29.8 %) of the 57 taxa that exist in Mexico. The most studied species, in descending order, are: *P. pseudostrobus* Lindl., *P. greggii* Engelm. ex Parl., *P. hartwegii* Lindl. and *P. montezumae* Lamb. (Figure 2). These four species account for 52 % of the reviewed studies, although Perry (1991) points out that several Mexican pine taxa have a great potential for use in commercial tree plantations.



Especie = Species; *Número de estudios publicados* = Number of published studies

Figure 2. Published studies in scientific journals by species.

Most of the studied species are of economic importance. Among the taxa that were not considered are those of lesser economic importance but with ecological relevance or with any level of risk of conservation; for example, *Pinus attenuata* Lemmon, *P. contorta* Douglas ex Loudon var. *murrayana*, *P. jeffreyi* Balf., *P. maximartinezii* Rzed., *P. rzedowski* Madrigal et M. Caball. and *P. chiapensis* (Martínez) Andresen, which are some of the 20 taxa included in the Official Mexican Standard of at-risk species (Semarnat, 2010), and regarding which further research is required for purposes of forest restoration.

In addition, there are studies on conservation and restoration that include the issue of seedling quality. Notably, the vast majority of the studies are directed to species that grow in a temperate-cold climate (94.6 %); only 4 % and 1.4 % refer to species of semi-arid regions and tropical, respectively; however, most of the national species correspond to the first type of climate.

Morphological variables and indexes

The most commonly used morphological variables are diameter (61 studies) and height (59 studies) (Figure 3). The morphological indicators for the 17 studied taxa are shown in Table 1.

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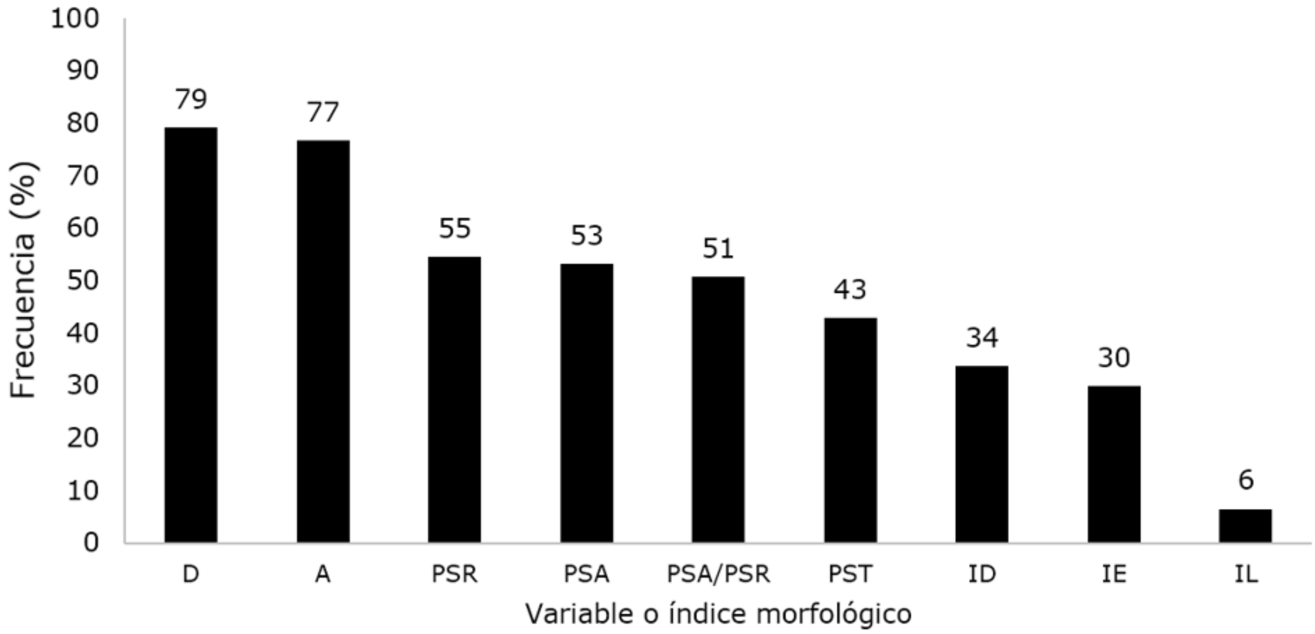


D = Diameter, *A* = Height, *PSR* = Root dry weight, *PSA* = Shoot dry weight, *PSA*/*PSR* = Shoot:root ratio, *PST* = Total dry weight, *ID* = Dickson´s Index, *IE* = Height:diameter ratio, *IL* = Lignification Index.

Figure 3. Ratio of found studies per morphological variable (total number of studies: 77, in 60 articles).

Table 1 Morphological variables and indices in certain species of the genus *Pinus*.

Species	PT	PP	Morphological variables and indexes								Ref.
			D (mm)	H (cm)	ADW (g)	RDW (g)	TDW (g)	ADW/RDW	SI	DI	
P. arizonica var. *cooperi* (C.E.Blanco)Farjon	C	8-11	3.0-4.8	10-25	1.6-2.3	0.7-1.2	2.3-3.7	1.6	2.2	0.9	1,2,3,4
P. ayacahuite Ehrenb. ex Schltdl.	BP	8	6.6	28.4	7.5	3.0	10.5	2.7	5.6	1.6	5
P. cembroides Zucc.	C	9.5	4.8	19.3	5.7	4.8	10.5	1.2	4.0	2.1	6
P. devoniana Lindl.*	C	9-12	7.1-13.5	8.6-11.2	3-10.6	1.2-3.3	4.2-13.9	0.4-3.2	2-12.2	1.2	7,8,9
P. devoniana Lindl.*	BP	8	15.9	9.8	10.5	3.7	14.2	2.8	6.3	2.5	5
P. douglasiana Martínez	C	7-12	3.3-5.0	13.3-35.3	5.3	1.1	6.5	3.1-3.6	4.2-6.1	0.2	8,9,10
P. durangensis Martínez	C	-	3.3	9.6	-	-	4.5	1.1	-	-	11
P. engelmannii Carrière*	C	7.5-12	4.4-6.5	10.2	2.4-3.8	0.4-2.8	2.8-6.6	3.2-6.1	1.6-2.2	0.4-1.2	12,13,14,15
P. greggii Engelm. ex Parl.	BP	10	3.9	24.9	-	-	-	0.4	-	0.5	16
P. greggii Engelm. ex Parl.	C	7-14	3.5-9.2	26.7-36.6	2.3-15.8	0.6-5.3	2.9-21.1	2.6-4.2	3.3-10.4	0.2-0.4	7,8,17,18
P. hartwegii Lindl.**	C	12	5.2	4.3	1.5	0.9	2.4	1.7	0.8	1.0	19
P. leiophylla Schiede ex Schltdl. & Cham.	C	12	3.5-4.6	14.4-30.0	1.7-2.6	0.5-1.2	2.1	0.3	-	0.3	20,21,22
P. montezumae Lamb.*	BP	8	11.2	-	5.1	2.3	7.3	0.4	-	-	23
P. montezumae Lamb.*	C	9.5-10	8.1-11.4	-	4.0-4.8	1.2-2.7	5.2-7.5	1.8-3.7	-	-	24,25
P. oocarpa Schiede	C	-	3.9	34	-	-	-	4.3	9.8	-	8
P. patula Schiede ex Schltdl. & Cham.	C	7.5	3.2	20.3	1.1	0.9	2.0	1.3	6.4	0.3	26
P. pringlei Shaw	C	13	6.6	15.4	4.5	3.6	8.1	1.2	-	-	27
P. pseudostrobus Lindl.	BP	8	6.2	14.9	6.4	1.4	7.9	0.2	-	-	23
P. pseudostrobus Lindl.	C	9-12	3.8-6.5	24.3-29.4	1.2-7.5	0.3-2.3	1.4-2.3	2.9-3.4	4.6-7.6	0.4-1.3	7,28,29
P. pseudostrobus var. *apulcensis* (Lindl.) Shaw	BP	7.5	3.3	18.5	1.2	2.5	3.6	2.3	-	-	30

*Species with grass stage. **Occasionally with grass stage. PP=Production type (C= Container; PB = Polyethylene bag). PT = Production period (months); Ref. = Reference(s): 1 (Prieto *et al*., 2004b); 2 (Prieto *et al*., 2007); 3 (Prieto *et al*., 2012); 4 (Prieto *et al*., 2018); 5 (Muñoz *et al*., 2015); 6 (Gutiérrez *et al*., 2015); 7 (Sáenz *et al*., 2014); 8 (Rueda *et al*., 2012); 9 (Bernaola *et al*., 2016); 10 (Rueda *et al*., 2014); 11 (Arteaga *et al*., 2003); 12 (Ávila *et al*., 2014); 13 (Rosales *et al*., 2015); 14 (Martínez *et al*., 2015); 15 (García *et al*., 2015); 16 (Barajas *et al*., 2004); 17 (Martínez *et al*., 2012); 18 (Sánchez *et al*., 2016); 19 (Bernaola *et al*., 2015); 20 (Palacios *et al*., 2017); 21 (Palacios *et al*., 2015); 22 (Buendía *et al*., 2017); 23 (Aldrete *et al*., 2002); 24 (Hernández *et al*., 2014); 25 (Aguilera *et al*., 2016a); 26 (Romero *et al*., 2012); 27 (López *et al*., 2018); 28 (Aguilera *et al*., 2016b); 29 (Ávila *et al*., 2017); 30 (Reyes *et al*., 2005). Note: The indicated intervals include the best recommended values in the references provided for each variable or index.

The review revealed other morphological variables and indices that were used to a lesser extent, such as: length of the main root (Ortíz and Rodríguez, 2008; Ávila *et al*., 2014; Robles *et al*., 2017), relative aerial dry weight and relative root dry weight (Sosa and Rodríguez, 2003; Robles *et al*., 2017), height/length of the main root ratio (Pineda *et al*., 2004; Sáenz *et al*., 2014; Muñoz *et al*., 2015), number and length of the root branches (Pineda *et al*., 2004), and volume of the root or shoot (Prieto *et al*., 2009; Bernaola *et al*., 2015, 2016; Prieto *et al.*, 2018). As for the morphological indexes, the Root Container Index (ICR) is cited on two occasions (Bernaola *et al*., 2015, 2016). This is estimated by dividing the volume of the container (cm^3) by the root volume (cm^3). According to the last citation, the higher the value of the ICR (27.5 and 125.0), the higher the survival rate (13 and 94 %, respectively) in *P. hartweggi* plants two years after establishment in the field.

Diameter. Used in 79 % of the studies. A plant with good diameter is more likely to have adequate lignification, carbohydrate reserves, a greater amount of buds for resprouting, and a more developed root system (Rodríguez, 2008). For Mexal and Landis (1990), of all attributes, this is the most relevant, because it defines the robustness of the stem that is associated with the strength and the survival of the plantation. According to Sáenz *et al*. (2014), specimens produced in tubes or polystyrene trays with a diameter >5 mm are more resistant to bending and tolerate the damage caused by pests better, although this may vary depending on the species. However, these trends are not universal (Grossnickle, 2012).

Different diameters were observed for different taxa, according to the production period and technology in the nursery. Some examples of production in a container are *P. hartwegii* aged 12 months, with 5.1 mm (Bernaola *et al*., 2015); *P. ayacahuite* Ehrenb. ex Schltdl. aged 8 months, with 6.6 mm (Muñoz *et al*., 2015); and *P. devoniana* Lindl. aged 7 months, with 4.2 mm (Rueda *et al*., 2014). In the case of seedlings produced in polyethylene bags: *P. montezumae* aged 8 months with 11.2 mm (Aldrete *et al*., 2002) and 24 months, with 15.1 mm (Robles *et al*., 2017).

This information is for reference only, because the caliper is a feature that varies for the same species, due to such factors as the type and capacity of the container used (Bernaola *et al*., 2015), the source of the seeds (Ortega *et al*., 2003), the presence of micorrhyzae (Martínez *et al*., 2015), and the type of substrate (Arteaga *et al*., 2003), whether a shade mesh is utilized and the amount of time during which it is left over the crop, or whether or not it is a grass stage species, among other factors. The more adverse the plantation site is (with a higher moisture limitation, southern exposures, poor or degraded soils, etc.), the higher the value of this indicator should be, and vice versa: in more productive sites, it will tend to be lower. With bare rooted *P. taeda* L., South *et al*. (1985) found that the likelihood of mortality in the field above 75 % was 88 % in trees with a caliper of root neck of < 2.4 mm; however, as the caliper increased, the likelihood diminished, 8 % in those with a caliper of > 6.3 mm.

Height. The next most commonly utilized attribute was height, in 77 % of the studies. This feature has been used as a predictor of the quality of a plant, because it is a general measure of its photosynthetic capacity and transpiration, which is reflected in the development of the root structure and which eventually results in a better utilization of nutrients, water and anchoring (Mexal and Landis, 1990). A good height allows the plant to compete for growing space and to capture more solar radiation, compared to smaller plants (Grossnickle, 2012).

However, in adverse sites taller trees may have lower survival; while in favorable places these individuals achieve better survival rates. For example, Tuttle *et al*. (1988) found that, after 2 years of having been planted in productive sites, *P. taeda* trees exhibited an increased survival with respect to their height, up to 98 % in 35 cm tall specimens; conversely, in unfavorable sites, their mean survival was 55 %. Of course, for grass stage species, height is not a useful indicator.

P. pseudostrobus produced in containers records heights of 27.9, 24.3 and 29.4 cm at 9, 10 and 12 months, respectively (Sáenz *et al*., 2014; Aguilera *et al*., 2016b; Ávila *et al*., 2017), and *P. hartwegii*, heights of 16.11 and 34.8 cm, at 12 and 24 months (Viveros *et al*, 2007; Bernaola *et al*., 2015). In the traditional production system in polyethylene bags, *P. greggii* has been observed to reach a height of 24.9 cm at 10 months (Barajas *et al*., 2004).

Dry weights and shoot:root ratio. The SDW was determined in 53 % of the reviewed studies, and the RDW, in 55 %. The reason for determining the weight on a dry matter basis is because the amount of water in the plant tissue can vary greatly, and therefore the fresh weight provides a less consistent measurement than the dry weight (Haase, 2007). As for the TDW, it is cited in 43 % of the studies. A quality plant should be as heavy as possible in order to produce the best growth, without losing the balance between the shoots and the roots required for its survival (Thompson, 1985). In addition, it has been proven that there is a strong relationship between the dry weight of plants and their stem diameter (Ritchie, 1984), and therefore, it correlates with the survival and growth in the field.

The shoot:root ratio provides more information than the individual dry weights and was determined in 51 % of the studies. A quality plant must have a low shoot:root ratio in order for its chances of survival to increase; since a well-developed root structure will allow a better absorption of nutrients, as well as a better accessibility to water. In this review we identified values ranging between 0.2, in *P. pseudostrobus* produced in bag (Aldrete *et al*., 2002), and 3.2 to 6.1, in *P. engelmannii* Carrière grown in containers (Rosales *et al*., 2015; García *et al*., 2015). Rueda *et al*. (2014) recommend a value of <2.0.

Dickson's Index. DI relates the variables diameter, height and shoot:root ratio, and adjusts them according to the size of the plant (TDW). Higher values represent a good, balanced development of the plant (Reyes *et al*., 2005). According to Rueda *et al*. (2014), plants with a DI > 0.5 are of good quality; in the present review, the obtained values ranged between 0.07 for *P. engelmannii* (Rosales *et al.*, 2015), and 4.7 for *P. montezumae*, a cespitous species, produced in a bag (Robles *et al*., 2017).

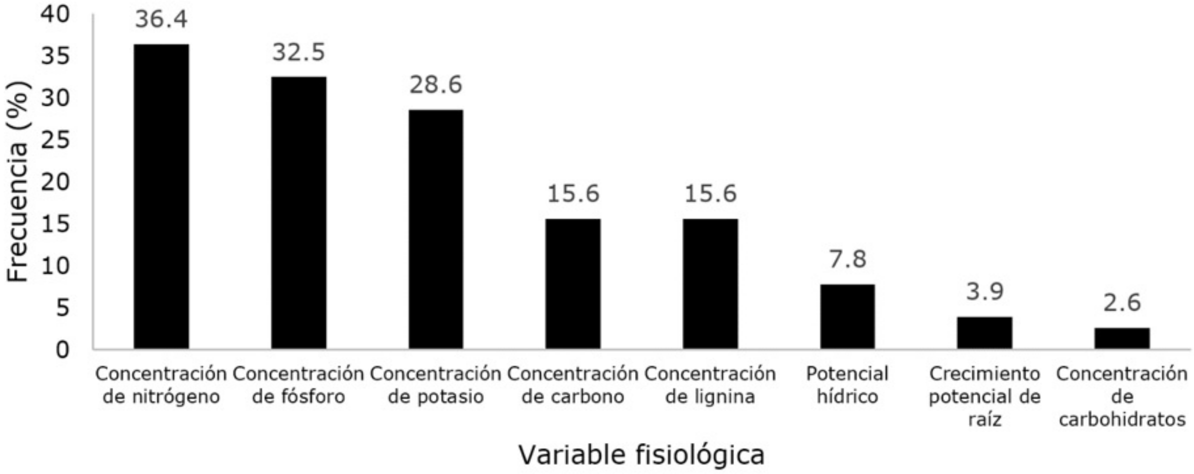
Slenderness Index. The SI is the relationship between the height and diameter of the plant; the lower its value, the lower and thicker the plant will be, and therefore, the more suitable for environments with limitations of moisture or cold (Rodríguez, 2008). Roller (1977) proved that a slenderness index below 6.0 is associated with a better quality of individuals of *Picea mariana*. In the consulted literature, the values ranged between 1.6 for *P. devoniana* at 7 months of age

(Rueda *et al*., 2014) and 10.4 for *P. greggii* at 9 months (Sáenz *et al*., 2014). If the index is high during the cultivation, aerial pruning is recommended; it is equally recommended when the shoot:root ratio has high values, in order to compensate transpiration with water absorption.

Lignification Index. The lignification index, cited in 6 % of the studies, provides an estimate of the degree of robustness that is needed for the plant to resist the stress, including hydric stress, at the planting site (Prieto *et al*., 2009). Values of 29.2, 22.9 and 24.3 % were registered for *P. engelmannii* when assessing the reduction in moisture availability as a form or preconditioning (Prieto *et al*., 2004a; Ávila *et al*., 2014); in *P. leiophylla* Schiede ex Schltdl. *et* Cham., the value was 30.9 %, considering the substrates and the nutrient addition rates (Buendía *et al.*, 2017). Although this index can be a useful estimate, the wet weight of a plant is not a very accurate measure, as we mentioned before.

Physiological variables

Concentration of nutrients. The study of the concentration of nutrients has focused mainly on nitrogen (36.4 % of the total of studies), phosphorus (32.5 %), and potassium (28.6 %) (Figure 4) (Table 2). It is important because it assesses one of the most critical components in high-quality nursery production: fertilizers (Jacobs and Landis, 2009). According to these authors, plants require adequate amounts of nutrients in order to keep the balance of their basic physiological processes, like photosynthesis, and to promote rapid growth and development. Without a good supply of nutrients, growth becomes slow, which reduces the vigor of the plant. A good fertilization promotes better growth rates. However, an excess of nutrients can affect the optimal development of individuals and even render them toxic; therefore, it is useful to measure the concentration of nutrients as an estimator of quality (Gutiérrez *et al*., 2015). Another negative effect of abundant fertilization with N, in the area of superfluous consumption, is that it promotes the synthesis of cytokinins, leaf development, and a low root development, as well as limited availability of carbohydrates; as documented by Rodríguez *et al*. (2002) for the bare-root production of *P. palustris* Mill. in the Southeast of the United States of America.



Frecuencia = Frequency; *Variable fisiológica* = Physiological variable; *Concentración de nitrógeno* = Nitrogen concentration; *Concentración de fósforo* = Phosphorus concentration; *Concentración de carbono* = Carbon concentration; *Concentración de lignina* = Lignin concentration; *Potencial hídrico* = Water potential; *Crecimiento potencial de raíz* = Potential root growth; *Concentración de carbohidratos* = Carbohydrates concentration

Figure 4. Frequency of the found studies per physiological variable.

Table 2. Some physiological indicators in *Pinus* species.

Species	PT	PP (meses)	N (%)	P (%)	K (%)	C (%)	Lignin (%)	Ref.
P. arizonica var. *cooperi* (C.E.Blanco)Farjon	C	8	1.5	0.2	1.5	-	-	1
P. ayacahuite Ehrenb. ex Schltdl.	BP	8	1.3	0.2	0.6	45.9	20.2	5
P. cembroides Zucc.	C	9.5	1.7	0.7	2.3	-	-	6
P. devoniana Lindl.	C	9-12	0.5-1.3	0.1-0.3	0.5-0.7	45.3-45.4	-	7,8,9
	BP	8	1.4	0.2	0.7	45.3	21.5	5
P. douglasiana Martínez	C	7	1.1-1.2	0.2-0.5	0.2-0.6	45.4-46.3	7.0-22.4	8,10
P. engelmannii Carrière	C	12	1.3	0.2	0.4	-	-	15
P. greggii Engelm. ex Parl.	C	9	1.0-1.3	0.2	0.6-0.7	45.9-46.0	21.5-25.6	7,8
P. hartwegii Lindl.	C	17	1.2	0.2	-	-	-	31
P. leiophylla Schiede ex Schltdl. & Cham.	C	-	2.3	0.2	0.5	-	-	22
P. montezumae Lamb.	C	9.5	1.2	0.2	0.3	-	-	25
P. oocarpa Schiede	C	-	1.4	0.3	0.6	45.3	18.5	8
P. pringlei Shaw	C	13	0.5	0.1	0.1	-	-	27
P. pseudostrobus Lindl.	C	9	1.0-1.1	0.2-0.3	0.6-0.6	45.2-45.9	20.0-20.4	8,32
Recommended values†	-	-	1.4-2.2	0.2-0.4	0.4-1.5	-	-	

PT = Production type (C=container, PB = Polyethylene bag). PP = Production period. Ref.=Reference(s). References: 31 (Ortega and Rodríguez, 2007); 32 (Gómez *et al*., 2013). †Standard values for nutrients in the leaf area of seedlings produced in containers (Landis, 1989). Note: The indicated intervals include the best values recommended in the references for each variable or index.

Each nutrient performs specific functions in the physiological processes of the plant; therefore, quantification of the nutrients helps establish its quality (Jacobs and Landis 2014). However, only four studies analyzed secondary macronutrients and micronutrients; notably, three of them refer to the use of ectomicorrhyzal fungi (Martínez *et al*., 2012; Rentería *et al*., 2017; Barragán *et al*., 2018); one evaluates the effect of two levels of pH of irrigation water, and two assess the effects of fertilization on the morphology and leaf concentration of nutrients of *P. cembroides* Zucc. (Gutiérrez *et al*., 2015).

In certain studies, the levels of N, P and K are below or above those proposed by Landis (1989) for conifers produced in containers. However, most of them declare a good plant quality, without visible responses of deficiency or excess of nutrients. This suggests that, in the face of the diversity of forest species in Mexico, the suggested concentrations of nutrients are a good starting point which, nevertheless, must be adjusted, often by reducing them.

Concentration of carbohydrates. Only two studies (2.6 %) were found for this variable in *P. greggii* (Cetina *et al*., 2001; Cetina *et al*., 2002). These authors compared the response to aerial and root pruning treatments, as well as to irrigation regimes, in the storage of soluble sugars and starch. Both studies emphasized the importance of the accumulation of NSC as reserves that are essential for survival, especially in the face of adverse conditions. The NSC play a role in the processes of energy transportation, metabolism and osmoregulation, and as a raw material in the synthesis of defense compounds and exchange with micorrhyzae in nutrient acquisition; and despite their prominence in the functionality of the plant, the understanding of the dynamics of their storage, controls and response to environmental stress are very limited; therefore, further research in this field is required (Marshall, 1985; Hartmann and Trumbore, 2016).

Water potential (Ψ_w). Used in 7.8 % of the studies. This indicator (whose numerical value is equal to that of hydric stress, but with the opposite symbol) is a measure of free energy or chemical potential of water and consists mainly of two components in a plant nursery: osmotic potential (Ψ_o) and the potential for pressure (Ψ_p) (Landis *et al*., 1989).

It is usually expressed in MPa. In general, a plant exhibits moderate to very high stress when its value corresponds to a range of -1.0 to -2.5 MPa (Ritchie *et al*., 2010). It is important to know the levels of stress that each species can tolerate, in order to avoid failure of the restoration programs. A clear example of this is the use of *P. leiophylla* as a pioneer species that can resist a stress of up to -3.5 MPa (Castelán, 2014).

Despite being a good predictor of drought resistance in field, the Ψ_w is very dynamic and can be affected by environmental conditions, the species and its provenance, the level of rest, the level of stress resistance and the age of the plant (Haase, 2007). Thus, the Ψ_w would reflect the quality of the plant only when stress is moderately high and is maintained during several days (Ritchie *et al*., 2010). Given this situation, there are other tests, such as the analysis of xylem susceptibility to cavitation, the stability of the cell membrane or photochemical efficiency, which may provide more information on the levels of water potential that a plant can attain before its physiological functioning becomes irreversibly damaged or affected.

Performance tests

Performance tests or performance attributes are determined by placing samples of plants in controlled environments and evaluating their specific responses. They have the inconvenience of being time consuming; however, they yield results in terms of the responses of the plant that often relate to its performance in the field (Ritchie, 1984). Resistance to cold and root growth potential, cited in only 1 % and 5 % of the studies, respectively, are among the most widely used tests in the world today.

Resistance to cold. It refers to the minimum temperature at which a percentage of a population of seedlings will survive or maintain a given level of damage (Ritchie, 1984). In temperate and cold areas, resistance to cold occurs naturally and is expressed through the hardening of the plant; however, when seeking to produce the plant in a nursery, it is necessary to understand the process of resistance to cold in order to obtain a properly hardened material (Glerum, 1985).

The only case, among the revised articles, in which this variable was used was that of Ramírez and Rodríguez (2010), who applied different concentrations of potassium nitrate during the hardening stage to nursery-grown *Pinus hartwegii* plants, which reached an age of 13 months. The likelihood of damage by cold after a frost (-5 °C by 2 h), simulated in a controlled environment chamber, was modeled. The damage was determined visually, although there are other methods to assess the resistance to cold, such as the loss of electrolytes test (Dexter *et al*., 1932; McKay, 1992; Burr *et al*., 2001) and the measurement of chlorophyll fluorescence (Vidaver *et al*., 1988). Finally, some authors have shown the effect of cold directly in the field. An example is the case of Viveros *et al*. (2007), who used this modality to prove that there may be intraspecies variations in tolerance to cold.

Root growth potential. Only three studies used it (3.9 % of the total). Sánchez *et al*. (2016) transplanted *Pinus oaxacana* and *P. greggii* seedlings into pots with a capacity of 10 L, with a substrate of bark (70 %) and perlite (30 %). The specimens were kept during 28 days in the greenhouse and were watered every day; at the end of this period, 17 and nine roots, respectively, were registered. Ávila *et al*. (2017) used the same methodology, but during 40 days, with *P. oaxacana*.

On the other hand, Robles *et al*. (2017) transplanted *P. montezumae* seedlings into pots of 4 L with a different substrate and watered them every other day during one month. These authors correlated survivals above 80 % with an average of 55 new roots; it was concluded that the test is good for estimating survival based on the ability of the plants to issue roots, given that, in general, it conveys an idea of the functionality of various physiological systems (Ritchie *et al*., 2010). However, it has also been widely discussed, because its accuracy is restricted to species that produce abundant roots and grow under unlimiting environments (Simpson and Ritchie, 1997; Oliet *et al*., 2003; Ritchie and Landis, 2003).

Nursery factors

The seedling quality can be affected by many factors; therefore, the nursery grower should ensure an appropriate combination of biotic and abiotic factors in order to achieve a good seedling quality. In this regard, various researches have focused on assessing the direct effect of these aspects through the years. In this review, the recurrence of such variables as the type and size of the container, the substrate used, the applied fertilizers, the applied preconditioning, and others like the type of pruning and the use of micorrhyzae was notorious. In general, it was demonstrated that the use of containers of greater capacity improves the root developments and plant quality (Prieto *et al*., 2007; Bernaola *et al*., 2015). On the other hand, the substrate utilized in the different researches was peat moss, due to its ideal physicochemical properties (Li *et al*., 2009); however, it was observed that in most of the studies, the proposed alternative substrates produced similar or superior results to those of peat moss (Reyes *et al.*, 2005; Hernández *et al*., 2014; Aguilera *et al*., 2016a; Aguilera *et al*., 2016b). In addition, the usefulness of controlled release fertilizers and of preconditioning as a way of contributing to improve the quality of the plants prior to their establishment in the field was proven (Prieto-Ruiz *et al*., 2004a, 2004b).

Verification of in-field survival

The vast majority of the reviewed studies used only morphological parameters as indicators of the quality of a plant. This may be due to the difficulty of access to expensive equipment and instruments to perform the physiological tests, as well as to the need for trained personnel. Or else, there may be a lack of financial resources to cover the costs in the laboratories that carry out this kind of determinations.

The physiological indicators should not be considered without taking into account the morphological characteristics, for a plant may have good nutrition, but if its root system is not well developed, the likelihood that it will survive in an environment with moisture limitations is low (Rodríguez, 2008; Gutiérrez *et al*., 2015).

Only in 29.5 % of the reviewed articles (Table 3) was a survival test performed, and given that the definition of plant quality involves survival, the subsequent studies must carry out in-field verifications, which will provide them with greater robustness and scientific rigor.

* * *

Table 3. Peer-reviewed studies on the assessment of survival in the field.

Species	AP	EI	Treatments	Results	Ref.
P. arizonica var. *cooperi* (C.E.Blanco) Farjon	18	Yes	80 cm^3 and 170 cm^3 containers, irrigation (every 48, 96 and 168 h), contrasting planting sites	Significant planting site in terms of survival and growth in H. S 85.6 %	2
P. arizonica var. *cooperi, P. engelmannii* Carrière	13	Yes	Two quality categories (D and H)	In the 1st, S=67.5 % (D>6.5 mm); no differences in the 2nd. (S >90 %)	4
P. greggii Engelm. ex Parl.	12	Yes	Root and stem pruning, irrigation	The pruning of the stem stimulated growth but not S	33
P. greggii Engelm. ex Parl.	36	Yes	Effect of chemical root pruning with Cu	No differences in growth or S (97.8 %).	16
P. greggii Engelm. ex Parl.	21	Yes	Provenances/progeny (21 families)	Differences between families, one with S=46 %	34
P. hartwegii Lindl.	36	Yes	Fire (S and biomass increases), 2 plant qualities	Higher S (48.8 %) in unburnt areas	35
P. hartwegii Lindl.	6	Yes	4 types of prescribed burnings, 2 plant qualities	>S in control and prescribed burning in March	31
P. hartwegii Lindl.	12	Yes	Nurse plants and microsites at the time of the planting	No effect on S. >N, P, K and growth in H with *Lupinus*	36
P. hartwegii Lindl.	12	No	Capacity of the container	S=96 % in 5L containers (retransplantation)	19
P. leiophylla Schiede ex Schltdl. & Cham.	6	Yes	Phenolic foam blocks hydrated at the time of the planting	>70 % S with 616 cm^3 and 462 cm^3 phenolic foam	21
P. montezumae Lamb.	12	No	2 qualities, 3 sites	High quality (D > 6 mm) had > S (83.82 %)	37
P. montezumae Lamb.	22	Yes	2 qualities, 2 altitudes, 2 aspects	Northern exposure (S=88.6 %) surpassed southern exposure (83.3 %)	38
P. patula Schiede ex Schltdl. & Cham.	12	Yes	Burning treatment and H class	S= 92 and 94 % in burnt and unburnt localities	39
P. pringlei Shaw	10	No	Inoculation with ectomicorrhyzal fungi	S=0 % without inoculation. Inoculated plant with 30-50 % S	27
P. pseudostrobus Lindl.	15	Yes	3 types of propagation, gullies	S=86 % with pine trees inoculated with *Pisolithus tinctorius*	32
P. pseudostrobus Lindl.	14	No	Production, preconditioning, site	S=52.9 %. Differences between production and sites	40

AP=Assessment period (months), EI=Evaluation of increases in morphological variables. Ref.=Reference, S=survival, D=diameter, H=height. References: 33 (Cetina *et al*., 2002), 34 (Díaz *et al*., 2012), 35 (Ortiz and Rodríguez, 2008), 36 (Ramírez and Rodríguez, 2009), 37 (Bautista *et al*., 2005), 38 (Robles *et al*., 2017), 39 (Sosa and Rodríguez, 2003), 40 (Sigala *et al*., 2015).

Conclusions

Most of the research uses morphological parameters —primarily height and diameter— as indicators of quality. Yet, although the diameter is a good indicator, the use of physiological parameters allows to better understand the operation of the plant both in the nursery and at the planting site.

There is room for further inquiry in relation to the physiological attributes, such as the reserves of carbohydrates, fluorescence of chlorophyll, and the loss of electrolytes, in the determination of seedling quality. In addition, performance tests such as the dormancy of the bud for species of cold environments, resistance to stress (due to drought or frost) and proof of root growth potential should be implemented more often.

In the future, more research must be supported with evidence of survival in the field in order to provide greater robustness and applicability to the results.

Few *Pinus* taxa are studied. It is important to deepen in the research that is yet to be carried out, but it is equally necessary to expand the range of species, if we are to have an impact and to incorporate restoration programs including taxa at risk and other taxa of economic or ecological interest.

The names of plant quality indicators need to be standardized in Mexico and in the Spanish language. In nursery studies, there is a recurrence of factors such as the type and size of the container, the utilized substrate, the applied fertilizers, and the applied preconditioning, among others. In general, it was observed that the use of containers of greater capacity improves the root development and plant quality. Peat moss is the most commonly used substrate in various researches; however, similar or superior results to those of peat moss were obtained with alternative substrates. The usefulness of controlled release fertilizers and of preconditioning as a way of contributing to improve the seedling quality prior to their establishment in the field has been proven.

Conflict of interests

The authors declare no conflict of interests.

Contribution by author

Sebastián Escobar-Alonso: collection, organization and analysis of the information; Dante Arturo Rodríguez-Trejo: research conception, organization and analysis of the information. All the authors participated in writing and implementation of the observations to the document.

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