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Original article

Reproductive capacity of *Pinus caribaea* Morelet var. *caribaea* Barret y Golfari in Marbajita´s the seed orchard

Capacidad reproductiva de *Pinus caribaea* Morelet var. *caribaea* Barret y Golfari en la unidad de producción de semilla mejorada Marbajita

Capacidade reprodutiva de *Pinus caribaea* Morelet var. caribaea Barret e Golfari na unidade de produção de sementes melhorada de Marbajita

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ABSTRACT

The study allows to evaluate the reproductive capacity of *Pinus caribaea* through the behavior of the reproductive indicators during two consecutive years. A sample of 109 cones was taken from trees in the improved seed production unit Marbajita-Cajálbana, corresponding to an area of natural pine forests in the municipality of La Palma. The characterization of their cones was made from their dimensions, besides determining the variables related to seed production such as fertile and infertile flakes, vain and full seeds, seed potential and efficiency, proportion of full and vain seeds, inbreeding index and germination percentage. Results related to cone length showed significant differences for the years evaluated, with the lowest values reported in 2016. High rates of inbreeding were evidenced above 12 %, acceptable for the species and for areas intended to produce improved seed. Germination was higher in 2017 with 100 %.

Keywords: Reproductive indicators; *Pinus caribaea* var. *caribaea*; Reproductive capacity.

RESUMEN

El estudio permite evaluar la capacidad reproductiva de *Pinus caribaea* a través del comportamiento de los indicadores reproductivos durante dos años consecutivos. Se tomó una muestra de 109 conos provenientes de árboles de la unidad de producción de semilla mejorada Marbajita-Cajálbana, correspondiente a un área de pinares naturales del municipio de La Palma. Se realizó la caracterización de sus conos a partir de sus dimensiones, además de determinar las variables relacionadas a la producción de semillas como escamas fértiles e infértiles, semillas vanas y llenas, potencial y eficiencia de semillas, proporción de semillas llenas y vanas, índice de endogamia y porcentaje de germinación. Los resultados relacionados a la longitud del cono mostraron diferencias significativas para los años evaluados, reportándose los valores más bajos en 2016. Se evidenciaron altos índices de endogamia por encima de un 12 %, admisible para la especie y para áreas que tiene la finalidad de producir semilla mejorada. La germinación fue superior en 2017 con un 100 %.

Palabras clave: Indicadores reproductivos; *Pinus caribaea* var. *caribaea*; Capacidad reproductiva.

RESUMO

O estudo permite avaliar a capacidade reprodutiva do Pinus caribaea através do comportamento dos indicadores reprodutivos durante dois anos consecutivos. Uma amostra de 109 cones foi retirada das árvores da unidade de produção de semente melhorada Marbajita-Cajálbana, correspondente a uma área de pinhais naturais no município de La Palma. A caracterização dos seus cones foi feita a partir das suas dimensões, além de determinar as variáveis relacionadas com a produção de sementes, tais como escalas férteis e inférteis, sementes vazias e cheias, potencial e eficiência das sementes, proporção de sementes cheias e vazias, índice de endogamia e percentagem de germinação. Os resultados relacionados com o comprimento do cone mostraram diferenças significativas para os anos avaliados, tendo os valores mais baixos sido reportados em 2016. Foram evidenciadas taxas elevadas de endogamia acima dos 12 %, aceitáveis para as espécies e para as áreas destinadas a produzir sementes melhoradas. A germinação foi mais elevada em 2017 com 100 %.

Palavras-chave: Indicadores reprodutivos; *Pinus caribaea* var. caribaea; capacidade reprodutiva.

INTRODUCTION

Pinus caribaea var. caribaea is one of the species recognized as a priority in the management and plantation within the development plans until 2030, due to its great plasticity of adaptation to different environments (García-Quintana *et al.,* 2013), as well as the quality of its wood for numerous uses. All this requires the increase of its plantations to obtain genetic quality seed, so the source of the material to be propagated is of special interest.

In this sense, in the 1960s work started on the selection and creation of seed sources for this species (García-Quintana *et al.,* 2007), based on genetic improvement programs by mass and individual selection, thus beginning research for the selection and improvement of the main seed sources.

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Seed orchards or stands are areas created or selected for the purpose of producing seeds with a certain degree of progress in the given characteristic, which is the object of forest improvement (White *et al*., 2007). Those are determined or selected according to morphological parameters, with economic interest; but the capacity of these trees and areas to produce quality seeds is rarely evaluated (Finkeldey *et al.,* 2007), so from a productive point of view it is not only important that they are superior specimens, but also producers of seed similar to the potential (Eriksson *et al.,* 2013).

P. caribaea var. caribaea has established seed production units, both by the method of mass selection (Meseta de Cajálbana) and individual (mainly: Malas Aguas, Estación experimental de Viñales), with the aim of increasing timber volumes.

Marbajita is one of the seed stands, in the Meseta de Cajálbana, was created in the 1960s from mass selection in a natural pine forest. During the 90's, more than 60% of its area was burned, so it was restored from superior trees of the same origin; it represents one of the main sources of seed for the species in Pinar del Río and for the country.

This stand reports the best behaviors in the nursery phase (technical reports, MINAG). On the other hand, the production of cones and seeds, as well as the quality certificates of the seed lots show a reduction, both in the quantity of seed and in the germination capacity.

Measures of reproductive characteristics, along with genetic diversity, can serve as indicators of reproductive and genetic status to determine and monitor the viability of populations at risk due to small population size (Delgado-Valerio *et al.,* 2013) and low density of trees within the forest (Quiroz-Vázquez *et al.*, 2017; Ramírez-Mandujano *et al.,* 2017).

Reproductive Indicators (RIs) are points of reference that provide qualitative or quantitative information on seed sources. They are also basic tools for monitoring populations (Flores *et al.,* 2012), especially those designed to meet the needs of forestry (Pérez-Reyes and Geada-López, 2020; Santos-Sánchez *et al*., 2018).

The objective of this study was to evaluate the reproductive capacity through the behavior of the reproductive indicators in a seed mass (Marbajita-Cajálbana) of *P. caribaea var. caribaea, i*n two years.

MATERIALS AND METHODS

Samples of 60 and 49 cones of the Marbajita-Cajálbana seed mass were collected in July 2016 and 2017. The seed production unit belongs to the UEB of the municipality of La Palma (Figure 1), presents the category of improved seed production and corresponds to an area of natural pine forest treated for this purpose. The seed stand occupies a total of 72 ha of pine forests, with an average height of 20-25 meters.

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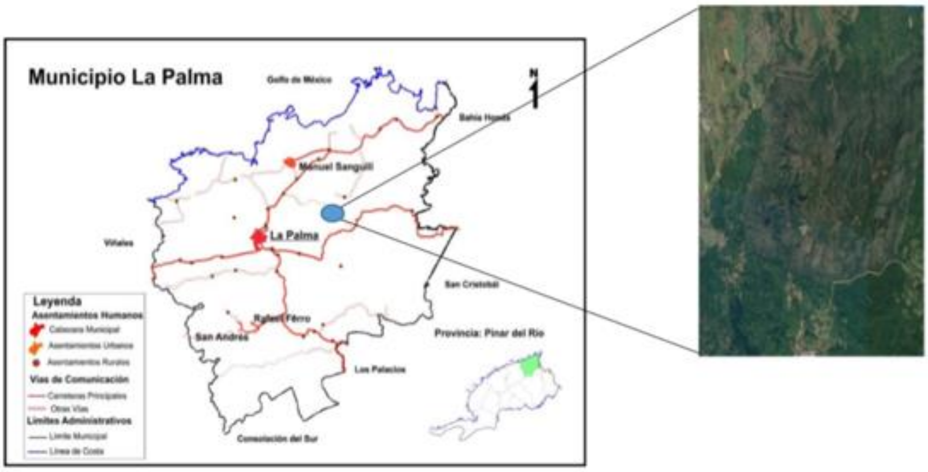

Figure 1. - Location of the Marbajita-Cajálbana seed stand

The cones were dried at room temperature for one month until the beginning of their opening and seed extraction. Their length was then determined and they were then dissected to count the Total Number of Flakes (NoE), in addition to Fertile Flakes (EF) and Infertile Flakes (Einf), Full Seeds (SLL) and Vane Seeds (SV). The seed certification test was carried out under Cuban Standard NC: 71-04/87.

The indicators were calculated using Bramlett's formulas (Quiroz-Vázquez *et al.,* 2017). To infer the existence of inbreeding in the seed mass, a germination test was carried out together with the evaluation of the viability of the seeds. The germination test was established in silica sand (glass containers), for 45 days. Germinated seed was considered to be seed whose radicle length exceeded the length of the embryo. Seed viability was determined from colorimetric tests.

Biometric Analysis: an analysis of non-parametric Kruskal-Wallis variance and a range comparison test were used to detect differences between collection years.

RESULTS AND DISCUSSION

Cone dimensions showed differences in both collection years (Table 1). This value is generally associated with seed availability and germination capacity, which indicates that trees with larger cone sizes produce larger seeds (Flores-López *et al*., 2005). Seed weight is also important, there is evidence in conifers where heavier seeds are associated with larger seeds, and therefore produce more robust seedlings at least during the first year of life (Flores-López *et al*., 2005). However, it has been found that cone and seed variables can vary between populations, between individuals and between regions, for species with a wider distribution (Boratynska *et al.,* 2005).

Although the collected strobile in 2016 were larger than those collected in 2017, the latter yielded the best indicators related to seed production, the average Seed Potential (SP), Fertile Flakes (EF) and developed seeds (Table 1).

Flores-López *et al*., (2012) and Quiroz-Vázquez *et al.,* (2017) propose that seed potential is an indicator related to the genetic structure of populations or areas of seed production, and this in turn represents the effectiveness of the process of pollination, fertilization and maturation of the seed (Fernando, 2014). In addition, it is the most evident expression of the effective population size that determines the proportion of pollen needed to make allogamy effective (Fernando, 2014; Flores-López, 2014).

Forest trees, under natural conditions, do not produce seeds regularly, but rather in variable periods of years, which can be from one to seven years or more; this characteristic is associated with some factors of the climate and maturity of the tree (Alba-Landa *et al.,* 2005). Differences between seed years have been reported in several conifers, such as *P. greggii* (Alba-Landa *et al.,* 2005), whose variations have been attributed primarily to abiotic factors such as humidity (rain), wind and drought events.

The indicators associated with full and vain seeds are significantly different between crops, but not in their proportions. One possible cause is associated with the absence of cross-pollination and fertilization, which affects the efficiency of the seed and is to be expected in small populations or in solitary trees. The results show that there is a high percentage of vain seeds; this value is usually recommended to be below 20 % (Sorensen, 2001; Ledig *et al.,* 2002) and for conifers in seed source areas it is accepted that it is around 12 % as an indicator of unaffected depression by inbreeding (Sorensen, 2001; Ledig *et al.,* 2002). The results in two years show that this value was higher than expected for seed-producing areas and higher than that obtained for the species in a non-certified seed stand (Pérez-Reyes, unpublished data).

In this sense, in the seed stand (Marbajita) to be evaluated, the differences obtained in the indicators and germination must be related to environmental factors such as drought and high temperatures in the months of April-June 2013 to 2016, which correspond to the years of development of female strobile its pollination and seed formation. However, there were no differences between the proportion of SLLP.

Table 1. - Behavior of reproductive indicators and cone during two harvests

Variables	2016	2017
Cone length (cm)	9,31^{b}	8,53^{a}
PS	225,13^{a}	243,34^{b}
ES (%)	40,16^{a}	45,92^{b}
SD	135,7^{a}	160,6^{b}
SLLP	0,030	0,029
SVP	0,15	0,14
IEND	0,33^{b}	0,30^{a}
SV (%)	33,1^{a}	34,6^{b}
SLL (%)	66,9^{a}	69,5^{b}
EF	112,5^{a}	121,6^{b}
Einf	43,08^{b}	37,8^{a}
G (%)	48	100

Note*: averages with different letters have significant differences at P < 0,05*

Owens *et al.,* (2005, 2008); Flores-López *et al*., (2005) report for Pinus and *Picea* a decrease in seed potential, which could be associated with inbreeding, especially for stands with small effective sizes, given the low effectiveness of morphological mechanisms to prevent self-crossing and given that genetic incompatibility mechanisms have not been developed in this taxonomic group (Bower and Aitken, 2007). It is recognized that large population sizes are a requirement for seed stands and should correspond to the 100/1000 rule (Frankham *et al.,* 2014), so as to ensure cross-pollination and the conservation of genetic variability, especially in the case of natural stands converted into seed orchards.

Seed efficiency is the most representative variable within seed production (Bramlet *et al.,* 1977; Mosseler, 2001). This indicator varied substantially between years, with the lowest value reported in 2016, indicating that there may have been reduced pollen availability. The low seed efficiency is generally attributed to four causes: low pollination, presence of lethal genes, insect damage (Bustamante-García *et al*., 2012) and low pollen availability with asynchrony in pollen release (Fernando, 2014); however, studies in tropical pines are scarce (Fernando, 2014) and do not allow to reach a regularity in the causes. The results obtained are similar to those recorded for *P. patula* (Alba-Landa *et al.,* 1999), although lower than species such as *P. greggii* (Alba-Landa *et al*., 2005) and *P. hartwegii* (Alba-Landa *et al*., 2003) in natural stands and higher than *P. oocarpa* (Isaza *et al.,* 2002) and *P. sylvestris* (Sivacioðlu and Ayan, 2008) for a seed orchard.

According to the development of the cone from flowering to formation and maturity of the cone, there are also losses of strobile, which were not considered in the study. Strobile losses can be up to 50 % or more of the seed harvest and the total seed production can be evaluated by combining the cone harvest life tables (Owens y Fernando, 2007; Fernando, 2014).

The inbreeding estimator (IEND) is extremely important from a silvicultural point of view; it reflects stand health, pollen availability and the amount of viable seeds to be produced (White *et al*., 2007). The results show relatively high values (≥12 %), for areas that are intended to produce improved seed. Inbreeding depression generates low percentages in germination, which in turn limits the production of vigorous seedlings and produces an increase in abnormal seedlings. The effects associated with inbreeding depression reduce the number of filled seeds and increase the number of vain seeds, production of weak, albino seedlings, with less ability to survive (Ramírez-Mandujano *et al.,* 2017). These results show that the behavior of this indicator was similar in the two years; however, germination was higher in 2017 with 100 % of seeds germinated, and in 2016 it only reached 50 %. This makes us think that it was not inbreeding that caused the low germination, but the possible low availability of pollen or favourable conditions during the long process of formation and maturation of the seed in the species three years (Fernando, 2014).

The year 2016 reports the worst reproductive indicators, corresponding to the lowest germination values, but no abnormal seedlings were detected in any year evaluated. This, together with the behavior of the reproductive indicators, indicates that the climate conditions during the years of formation, pollination and maturation were the causes of the marked differences between the years (Fernando, 2014). Therefore, monitoring studies of seed sources through indicators are necessary to correlate their changes with bioclimatic variables and to document the incidence of climate change on seed production.

A comparison of the two years showed a better performance of the indicators in 2017; however, this did not correspond to the results obtained for the length of the cone.

The differences obtained in the reproductive indicators could be related to climatic factors, such as the prolonged drought 2013-2015.

In 2016 the worst reproductive indicators are reported, which in turn corresponds to the lowest by hundreds of germination for the species.

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