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SEEDLING EMERGENCY AND BIOMETRY OF FRUITS AND SEEDS OF *Cariniana pyriformis* FROM THE MIDDLE MAGDALENA VALLEY, COLOMBIA

PRATO, A. I.; ZARATÉ, D. A.; ZULUAGA, P. J. J.; GARZON, S. L. C. Seedling emergency and biometry of fruits and seeds of *Cariniana pyriformis* from the middle Mgdalena Valley, Colombia. **CERNE**, v. 25, n. 2, p.221-229, 2019.

HIGHLIGHTS

Cariniana pyriformis shows a higher variability in fresh mass per fruit (35%).

One kilogram corresponds to 6715 seeds with 11% humidity.

The application of GA_3 allows accelerating and synchronizing seedling emergence.

Immersion for 24 hours in 1000 mg·L^{-1} of GA_3 is recommended

Keywords:
Colombian mahogany
Germination
Gibberellins
Lecythidaceae

ABSTRACT

Aiming at contributing to the knowledge of the biology and propagation of *Cariniana pyriformis* Miers, a native species with high timber and ecological attributes, ripe fruits were harvested. Initially, in 400 seeds and 320 fruits, the size, fresh mass, seed water content, number of seeds per fruit, number of seeds per kilogram and fruit color were assessed. Then, a homogenous seed sample was immersed for 24 hours in five doses (0, 250, 500, 750 and 1000 mg·L^{-1}) of gibberellins (GA_3) and cultivated in a greenhouse. For this, a completely randomized design was adopted with six replications of 25 seeds each. Fruits and seeds showed values, on average ± standard deviation, of 76.1 ± 11 mm and 48.1 ± 8.2 mm in length, and 49.6 ± 5 mm and 7.2 ± 0.7 mm of width, respectively, with 62.3 ± 22.3 g of fresh mass per fruit and 13 ± 3.1 seeds per fruit. One kilogram corresponded on average to 6715 seeds with 11% water content. In the greenhouse, emergence speed index and emergency percentage of seedlings increased as the GA_3 dose increased, allowing a more concentrated emergency process in a shorter period. Highest variation was found for fresh mass per fruit (CV= 35%) and number of seeds per fruit (CV= 24%). Immersion in 1000 mg·L^{-1} in GA_3 for 24 hours triggers seedling emergency in *C. pyriformis* (approx. 71%), and then it is recommended for plant propagation, since the treatment accelerated and synchronized seedling emergency.

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INTRODUCTION

Abarco, chibugá, piloncillo, bacú or Colombian mahogany (*Cariniana pyriformis* Miers) is a forest species belonging to the Lecythidaceae family and Lecythidoideae subfamily (Huang et al., 2015; Cárdenas et al., 2015). This subfamily is restricted to tropical regions of the Western Hemisphere and includes 11 genera and 210 described species, with the Amazon basin and non-flooded lowland forests of the Guianas as the center of diversity (Huang et al., 2015). Particularly, the genus *Cariniana* includes 17 species, eight of which occur naturally in Brazil (Smith et al., 2015).

C. pyriformis is a native tree species that occurs in Northwestern Colombia, Eastern Panama and Maracaibo Lake Basin in Venezuela (Mori et al., 2017). The high wood strength and durability make this a promising species for forestry and agroforestry systems (AFS) associated to cacao, depending on the climatic conditions of a particular region (Agudelo - Castañeda et al., 2018; Suarez et al., 2018). The species is categorized as Critically Endangered, being the reason why some municipalities in Colombia maintain a ban on a ban on commercial logging (Cárdenas et al., 2015). In addition to its high timber attributes, abarco has the potential to be recommended in ecological restoration programs, just as the species *Cariniana legalis* (Mart.) Kuntze and *Cariniana estrellensis* (Raddi) Kuntze that have been used in coffee AFS in Brazil (Karsten et al. al., 2014; Nicodemo et al., 2016).

Apart from some studies carried out in certain geographical areas of Colombia, e.g. Antioquia, Choco and Córdoba (Betancur and Raigosa, 1973; Diez and Moreno, 1998; Gómez, 2010; Espitia et al., 2017), information about fruit and seed morphology is scarce for this species. These aspects are crucial since they are related to dispersal strategies, establishment of seedlings in the field and detection of genetic variability in populations or individuals. In addition, it also contributes to studies on ecological succession and regeneration of forest ecosystems (Silva et al., 2013; Zhu et al., 2016; Cangussu et al., 2018).

Abarco seeds could be classified as intermediate orthodox, allowing their storage for extended periods according to Betancur and Raigosa (1973). However, the demand for seeds is difficult due to its suprannual fructification or even scarce in some years (Betancur and Raigosa, 1973; Gómez, 2010). Additionally, because of the constant decrease in number of adult individuals, the development of protocols that facilitate the propagation and massive production of seedlings is required.

According to the aforementioned, the aim of this study was to characterize the biometry and some physical properties of fruits and seeds of *C. pyriformis* from native trees of the Middle Magdalena Valley in Santander department (Colombia), as well as the effect of gibberellins application on seedling emergency.

MATERIAL AND METHODS

Study area and tree selection

During April and August 2016 in the rural area of the municipality of El Carmen de Chucuri, department of Santander (Colombia), mature fruits of four wild adult trees of *C. pyriformis*, known hereinafter as T1, T2, T3 and T4, were harvested when these initiated natural operculum opening (Table 1). The selection was made according to evidence of previous fruiting, good sanitary status and a minimum distance of 100 m between each tree to avoid related crosses. The limited access to private properties where some trees were located and reduction of the populations sampled in the region conditioned the number of adult individuals selected for this study.

Harvest was carried out with a sickle in the upper third of the crown. According to the Köppen classification system (Köppen, 1918), the region has an equatorial tropical climate (Af) with rainfall distributed in two periods, from April to May and from October to November, i.e. a bimodal regime. The average temperature and precipitation (of 20 years) is 24 °C and 2580 mm, respectively, according to the climatological station of Instituto de Hidrología, Meteorología y Estudios Ambiental (IDEAM), and to 10 km of distance from the study area.

Climatic information during fruit the formation period (October 2015 to August 2016) as well as the geographical location of trees were carried out during field visits, made every three weeks. Figure 1A and Figure 1B show the data recorded during fruit formation period (October 2015 to August 2016) and the geographical location of trees (for more detail on the phenological monitoring, please consult Pelaez et al., 2018).

One day after harvesting, fruits were transported to the facilities of the Research Center C.I. La Suiza of Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), located in the municipality of Rionegro, Santander (7°22'11.1 N; 73°10'39.2'' W; 530 m of altitude). Fruits were identified by tree and exposed to to the sun in sunny days and collected at sunset to facilitate seed extraction during two weeks (at a temperature

TABLE 1 Harvest date, altitude, plant height, diameter at breast height (DBH) and geographical coordinates of the *Cariniana pyriformis* trees selected.

Tree	Harvest date	Altitude (m)	Plant height (m)*	DBH (cm)	Geographical coordinates
T1	25/04/2016	350	30.3	45.3	6°40'50.79" N; 73°36'27.31" W
T2	25/04/2016	362	28.9	55.5	6°42'2.63" N; 73°34'21.01" W
T3	09/08/2016	245	29.5	79.3	6°46'32.25" N; 73°35'19.08" W
T4	09/08/2016	480	33.2	86.2	6°41'14.84" N; 73°36'44.16" W

* Height estimated using an inclinometer (Suunto Pm-5/360 P)

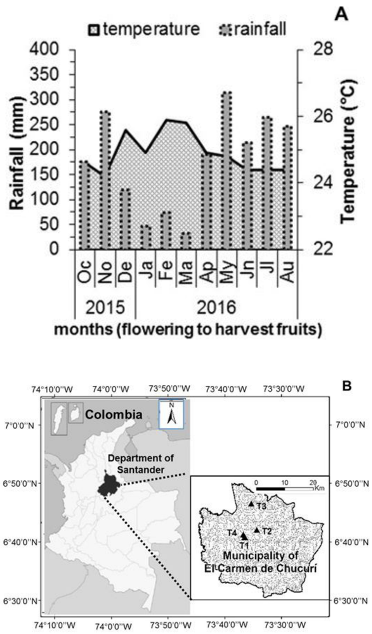

FIGURE 1 A) Precipitation (bars) and monthly temperature (polygon) during the fruit formation period, and B) geographical location of *Cariniana pyriformis* trees assessed.

of 24.5 °C and 90.4% relative humidity). These were placed inside well-ventilated environments to avoid rainfall. As seeds were released these were stored in a refrigerator inside plastic containers (at a temperature of 3 °C and 30% relative humidity). Subsequently, the following experiments were established.

Experiment 1: biometrics of fruits and seeds

The maturation state of the fruits was established by reflectance analysis in the red, green and blue spectra with a color analyzer (Lutron RGB-100), and images obtained were reproduced in a specific color reproduction software (Photoshop CS6). The resulting fruit value was the average of three points in the apical, intermediate and basal regions.

For each tree, 80 fruits without visual symptoms of diseases, plagues, physical damage (e.g. perforations), or that were broken were randomly selected per tree. The number of seeds per fruit, fruit fresh mass, fruit length and width in the operculum region were assessed. A precision scale (0.01 g) was used for weight recordings and a digital caliper (precision of 0.01 mm) was used for length measurements. The seeds considered in this trial had a mature stage, were intact, and without visual symptoms of pest attack.

The seeds water content was established by the oven method at 105 °C for 24 hours, using three replications of 2 g, weighed in a precision scale (0.01 g), and the results expressed as percentage (Brasil, 2009). The weight of 1000 seeds was established by the average value obtained with eight replications of 100 seeds using an electronic scale (0.01 g), being then calculated the number of seeds per kilogram (Brasil, 2009).

For each tree, 100 seeds were randomly selected, and their length (including its winged section), width, and thickness were measured with a digital caliper (0.01 mm). Moreover, wing width per seed was measured in its middle section.

A completely randomized experimental design with four treatments (trees: T1, T2, T3 and T4) and six replicates of 25 seeds per tree were employed. For fruits, four replications of 20 fruits per tree were used, and for fruit coloring, five replications of eight fruits per tree were employed. The biometric data of fruits and seeds were analyzed for their adherence to normality through the Shapiro-Wilk test, and its frequencies distributed in six class intervals, calculating means, minimums, maximums, standard deviations and the coefficient of experimental variation.

Experiment 2: Gibberellins and seedling emergency

From a homogenized seed sample of 300 g storage for 35 days in the refrigerator, the required seeds for experiment was disinfected with sodium hypochlorite (10% for 10 minutes) and washed three times with distilled water. Since fruiting of the individuals selected was not synchronous, the harvest obtained in August 2016 was selected. Subsequently, seeds (without wing) were immersed for 24 hours in five doses of GA_3 (0, 250, 500, 750 and 1000 $mg \cdot L^{-1}$) in plastic containers containing 100 mL of solution. The control treatment comprised immersion for 24 hours in distilled water. Seeds were planted in plastic boxes (12 cm x 12 cm x 3 cm, length x width x height) using washed and sterilized river sand (oven at 120 °C for 2 hours). Propagation was carried out in a greenhouse with an anti-thermal plastic cover that provides 50% shade. Irrigation was applied evenly to maintain adequate moisture in the trays (to approximately 60% of field capacity).

The number of normal emerged seedlings, when the cotyledons exceeded the substrate line, was recorded every two days until the stabilization of all the treatments, lasting 54 days after sowing (DAS). Seedling emergency (SE), mean emergence time (MET), emergence speed index (ESI) and relative emergence frequency (REF) were calculated according to the formulas described by Ranal and Santana (2006). In the experimental period, the minimum, maximum and mean temperature (19.5 °C, 41.6 °C and 26.6 °C) and relative humidity (41.5%, 100% and 87.8%) were recorded every 10 min using a data logger (CEM DT-171 ®).

A completely randomized experimental design with six replications of 25 seeds was employed, referring to five doses of GA_3 (0, 250, 500, 750 and 1000 mg·L^{-1}).

Statistical analysis

Data on biometric variables were subjected to an analysis of variance followed by the Tukey test ($p<0.05$) for the parametric results. When the normality and homogeneity of the variances were not verified by the Shapiro-Wilk and Levene tests, respectively, the nonparametric analysis (Kruskal-Wallis) was used, and followed by the Dunn test ($p<0.05$). The SE, MET and ESI variables were subjected to polynomial regression analysis up to a third grade. When the F-test indicated a 5% probability of significance for more than one regression, the equation of higher significance and the coefficient of determination was selected. All the analyzes were carried out using the statistical program R version 3.3.2 (R Core Team, 2017).

RESULTS

Experiment 1: biometrics of fruits and seeds

There were significant differences for all the morphometric variables of fruits and seeds among the trees. Only wing ($p< 0.51$) and seed width ($p< 0.11$) showed a normal distribution (Table 2, Table 3, and Figure 2).

Fruit morphometry showed wide variability for fresh mass (CV= 35.7%), with a predominance between 40 and 60 g (40%) and from 60 to 80 g (22.5%), with an average of 62.3 g (Figure 2A). The lowest value was recorded in the T3 tree (mean = 38.8 g) and the highest in T2 (mean= 94.3 g). However, there was a slight variation for fruit length with the majority inserted in classes 7 and 8 cm (35.4%), and in 8 to 9 cm (23.2%), with an average of 7.6 cm (Figure 2B). Fruit width had a predominance between 4.3 to 5.3 cm (61.6%) and an average of 5 cm (Figure 2C). These two dimensions showed a coefficient of experimental variation of less than 15%.

Mature fruits of abarco were characterized by their dark brown coloration (Table 2). The number of seeds per fruit had a high coefficient of variation (24.2%), predominating between 11 to 14 seeds (38.5%) and with an average of 13 seeds (Figure 2D). The T2 tree had the highest number of seeds per fruit (15).

Seed dimensions also showed similar values (Table 3 and Figures 2E, F, G and H). Mean length was 48.1 mm and there was a predominance between 37 and 51 mm

TABLE 2 Means of fresh mass (MF), length (LF), width (WF) and RGB colors of fruits in four adult *C. pyriformis* trees.

Tree	MF (g) ^{B}	LF ^{A} (cm)	WF ^{B} (cm)	Reflectance values * R^B	G^B	B^B	Color
T1	55.7 bc	7.3 c	4.6 b	25.9 bc	20.8 bc	17.5 bc	
T2	94.3 a	8.9 a	5.5 a	25.7 c	20.4 c	17.1 c	
T3	38.8 c	6.2 d	4.5 b	37.3 ab	30.4 ab	25.8 ab	
T4	60.5 ab	8.0 b	5.2 ab	46.6 a	35.7 a	28.5 a	
Mean	62.33	7.61	4.96	33.9	26.8	22.2	

RGB: * Reflectance values in the red, green and blue spectra employing a color analyzer (Lutron PCE-1002). Means with the same letter in the column do not differ significantly ($p < 0.05$) from each other according to Tukey (A) and Kruskall-Wallis (B) tests.

(59.4%); average width presented a value of 7.2 mm with a predominance ranged between 7 to 8 mm (51.1%); and average thickness showed a value of 4.4 mm and was predominantly between 4 and 4.8 mm (45.3%). Furthermore, dimensions were higher in the T4 tree and smaller in the T1 compared to the others. As for wing width values, the average was 13.1 mm and predominance ranged between 11 and 14 mm (38.5%). The coefficient of experimental variation for seed dimensions was between 10 and 17%. An average of 6715 seeds per kg was found, and seeds had 10.9% water content, which corresponds to a harvest of 523 fruits.

Experiment 2: Gibberellins and seedling emergency

All the physiological variables evaluated had a significant effect due to the application of GA_3 (Figure 3). Seedling emergency showed a positive linear dependence ($R^2= 0.81$, $p<0.05$, Figure 3A) in relation to increasing doses of GA_3, obtaining a maximum of 73.5% when using 750 and 1000 mg·L^{-1} (Figure 3A). However, seed germination in absence of a phytoregulator had a value of 51.1%. In addition, increasing doses of GA_3

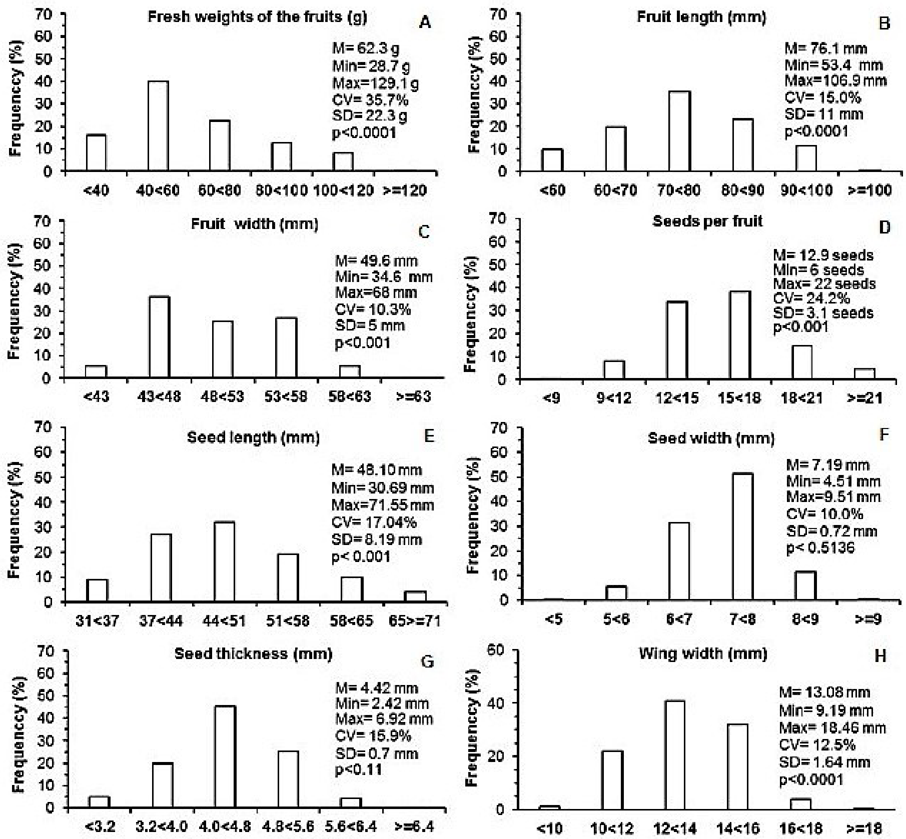

FIGURE 2 Frequency distributions in six intervals, mean (M), minimum value (Min), maximum value (Max), coefficient of variation (CV), standard deviation (SD) and level of significance of the Shapiro-Wilk normality test (p) for fresh mass per fruit (A), fruit length (B), fruit width (C), seeds per fruit (D), seed length (E), seed width (F), seed thickness (G) and wing width (H) of *C. pyriformis*. n= 320 fruits and 400 seeds.

TABLE 3 Means of seeds number per fruit (NSF), seeds number per kg (NSK), seed length (LS), width (WS) and thickness (TS), wing width (WWS) and seed water content (SWC) for four *C. pyriformis* trees.

Tree	NSF [B]	NSK *	LSA	WSA	TS A	WWS	SWC
	(number of seeds)			(mm)			(%) *
T1	11.9 b	6224.5	40.10 b	6.71 b	3.83 c	13.75 a	13.4
T2	15.0 a	6289.4	48.74 d	7.45 a	4.33 bc	12.54 b	11.6
T3	12.0 b	7568.3	44.99 c	7.13 ab	4.43 b	12.40 b	9.4
T4	13.3 ab	6778.1	57.14 a	7.48 a	5.04 a	13.80 a	9.1
Mean	13.0	6715	47.74	7.19	4.41	13.1	10.9

Means with the same letter in the column do not differ significantly ($p < 0.05$) from each other according to Tukey ([A]) and Kruskal-Wallis ([B]) tests. * No statistical analysis was carried out

decreased the MET, observing a lineal behavior (R^2= 0.97, $p<0.01$, Figure 3B), which means a reduction of up to 10 days when the 1000 mg·L^{-1} dose (27 days) was used, compared to the control treatment. This positive influence of GA_3 was confirmed by the ESI that showed a linear response (R^2= 0.81, $p<0.05$, Figure 3C), reaching a maximum of 6.01 with the dose of 1000 mg·L^{-1}. In contrast, the other treatments had lower values than the 1000 mg·L^{-1} treatment.

In general, seedling emergency comprised a period from 16 to 50 DAS, with marked differences in REF according to the dose (Figure 3D). With the exception of treatments 750 and 1000 mg·L^{-1} that showed a polygon with unimodal character and a more homogeneous emergency process, the other treatments were characterized by being polymodal and with several peaks, between two to four.

The mean of this trial had a MET of 32.5 days. Consequently, the MET values for 0 (37.6 days) and 250 (35.1 days) mg·L^{-1} of GA_3 moved to the right of the polygon, observing a large proportion of seedlings (21%) emerging between 36 and 40 DAS (Figure 3D). Furthermore, there was also a peak of lower intensity between 44 and 48 DAS, where up to 10% of the seedlings emerged. On the contrary, the doses of 750 (30 days) and 1000 (27.4 days) mg·L^{-1} of GA_3 showed a peak between 22 and 26 DAS of 38% of seedlings emerged, i.e., to the left of the polygon. Finally, in these two doses, the emergency decreases until the end of the process.

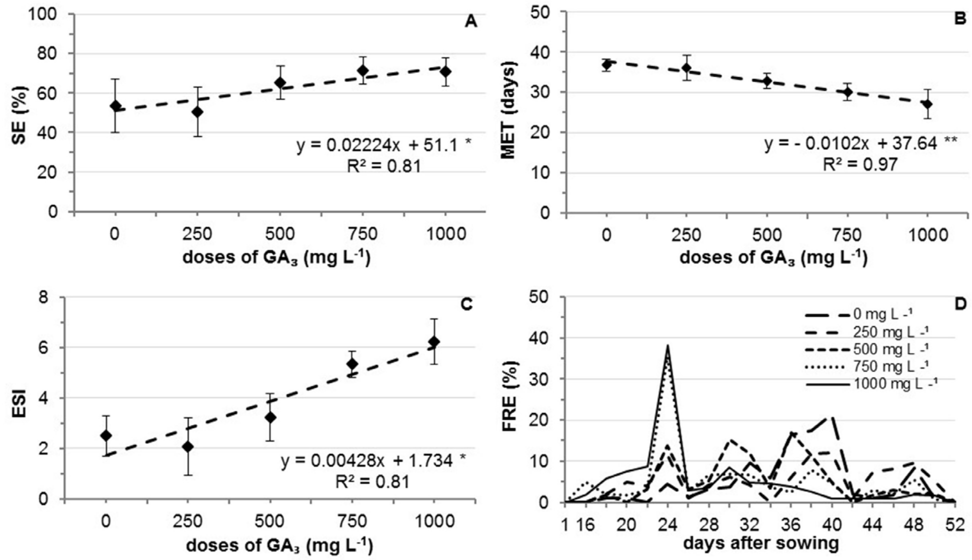

FIGURE 3 Means for A) seedling emergency (SE), B) mean emergence time (MET), C) emergence speed index (ESI), and D) relative emergence frequency (REF) for *Cariniana pyriformis*. *, ** significant at 5% or 1% for the F-test, respectively. Vertical bar indicates vertical bar indicates desviation standard.

Thus, higher doses of GA_3 induced a smaller distribution of emergency over time.

DISCUSSION

A few studies in the literature on fruit/seed biometry of abarco found similar results in comparison to our study, despite being carried out in different biogeographical regions of Colombia. For the of the Middle Magdalena Valley region in the municipality of San Luis Antioquia (Antioquia), Diez and Moreno (1998) found that fruits (6 to 8 cm of length, 5.5 cm of width and 10 to 25 seeds per fruit) and seeds (4 cm of length, and 6.6 cm of width) had similar values to the ones found in the current study. The number of seeds per kilogram was 6237. For the Lower Chocoan Atrato-Urabá region in the municipality of Riosucio, Betancur and Raigosa (1973) found that the dimensions of fruits and seeds were almost identical.

As an exception, in the Sinu River Basin region in the municipality of Tierralta, Córdoba, Espitia et al. (2017) found that the number of seeds per kilogram varied abruptly, i.e. between 7082 up to 31390 and with an average of 19498 seeds per kilogram. The dimension differences observed could indicate the interaction between environmental factors and genetic diversity, as well as the maturity state of fruits and their development process during harvest, since they can vary between and within trees, or between years for the same biogeographic zone (Silva et al., 2013; Bezerra et al., 2012; Cangussu et al., 2018).

The *Cariniana* genus has evolved an anemochoric dispersion mechanism (Prance and Mori, 1978). According to the average dimensions observed in the current study (width of 49.6 mm and length of 76.1 mm), the seeds of the individuals of *C. pyriformis* sampled could be classified as small, elongated and of low weight, factors that contribute to their dispersion. In fact, the characteristics of the diaspore (shape, mass and morphometry) can have a relative effect on its mobility, with wind speed and tree height being the most important factors determining the dispersion distance (Zhu et al., 2016).

Initially, abarco seeds registered an average water content of 10.9% and a variation between 9.1 and 13.4% (Table 3), which could confirm its intermediate orthodox behavior as reported for this species by Betancur and Raigosa (1973). The authors confirmed that abarco seeds conserve much better their germinative potential when stored at 4 °C for 30 days (72%) as opposed to 20°C for 90 days (35%). Namely, from 60 days and under environmental temperature conditions, loss of seed viability begins. Seeds of *C. legalis* and *C. estrellensis*, other species of the *Cariniana* genus might also be classified as orthodox (Azevedo, 2009; Gómez, 2010; Kopper et al., 2010). However, it is necessary to deepen further the ideal conditions to store abarco seeds for longer periods in different environments and packaging.

Mosquera et al. (2012) and Espitia et al. (2016) found a germination percentage in abarco of 75 and 70%, respectively, in seeds immersed for 24 hours in water and cultivated with sand substrate. The values were superior to the control treatment found in this study (51.1%), demonstrating the high variability of the results under similar germination conditions for this native species.

The use of GA_3 allowed emergency percentage to increase from 51.1 to 73.5% and decrease the MET from 37.6 to 27.4 days with immersion using the highest dose (Figure 3), and revealing its usefulness. That is, although a value of 51.1% without applying a phytoregulator could be considered acceptable, the results observed are important since seed supply may be compromised at certain times of the year as mentioned above. In addition, a long germination time makes forest seeds more susceptible to pathogen attack. According to Piveta et al. (2014), a shorter period decreases losses in the nursery, even once the plantation has been established. For *C. pyriformis* seeds harvested in forest areas of the department of Córdoba, the presence of seven genera of fungi was identified ranging between 1 and 77% of external incidence (Campo et al., 2014). This leads to unviable commercialization.

C. pyriformis is a native species that demonstrates its ability to adapt to a changing environment showing a higher delay and heterogeneity in seedling emergency process, evidenced in the control treatment. This behavior increases the chances of finding more favorable conditions for seedling establishment. Furthermore, this type of relative dormancy, common in native species, ensures that at least part of the seeds germinate and develop (Berger et al., 2014). According to the classification described by Baskin and Baskin (2004) and based on the fact that there was permeability in the seed cover to the phytoregulator, it is suggested with the current study that a certain amount of abarco seeds might have seeds might have non-deep physiological dormancy: GA_3 promotes germination. Further, other separate or combined pregerminative treatments, such as warm moist stratification or alternation of temperatures to break dormancy in abarco, should be evaluated because these treatments have proven to be effective with this type of dormancy in other forestry species (Pipinis et al., 2014; Cabello et al., 2019).

From a nursery perspective, the main interest is to increase the emergency speed and synchrony processes, because it facilitates the tasks of selecting and transplanting uniform seedlings, increasing the efficiency of the production cycle. Clearly, the dose de 1000 $mg \cdot L^{-1}$ generated a slightly higher emergency compared to the dose of 750 $mg \cdot L^{-1}$ contributing to this goal, meanwhile the other treatments resembled more closely the control.

There was no detrimental effect with the doses evaluated for this study. Piveta et al. (2014) verified that increasing immersion time of *Lithraea molleoides* (Vell.) seeds in GA_3 increased germination using a low concentration (48 hours and 250 $mg \cdot L^{-1}$) than the opposite (24 hours and 500 $mg \cdot L^{-1}$). In *Vitex montevidensis* Cham, another species of forest and ecological interest, Malavasi et al. (2011) concluded that the application of 200 $mg \cdot L^{-1}$ of GA_3 for 47 hours allowed increasing the germination from 19 to 56%.

In the future, it would be convenient to evaluate the combination of times and GA_3 concentrations in abarco seeds, in addition to finding the optimum maturity state of fruits, storage conditions and times, pre-germination treatments, among other factors. No published information on the use of GA_3 or other growth regulators in this species was found, so the knowledge continues to be scarce.

CONCLUSIONS

Major variability in the fruit fresh mass and number of seeds per fruit in abarco was verified in this study; furthermore, significant differences in all the biometric variables assessed among seeds and fruits of mother trees was evidenced. Mature abarco seeds seem to have non-deep physiological dormancy. Therefore, the application of gibberellins at a dose of 1000 $mg \cdot L^{-1}$ for 24 hours is recommended because it increases, accelerates and synchronizes the seedlings emergency of reaching 71%.

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