Technical note

Seed characteristics of five mulberry (*Morus alba* L.) varieties harvested in Matanzas, Cuba♦

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Abstract

The objective of the study was to characterize the morphological and physiological traits of the seeds of five *Morus alba* L. varieties (cubana, tigreada, universidad, universidad mejorada and yu-62), which were harvested at the Pastures and Forages Research Station Indio Hatuey –Matanzas, Cuba–. A completely randomized design was used, and the type of embryo, seed size and mass, moisture content, allocation of biomass to the seed reserves (embryo-endosperm) and desiccation tolerance index, were determined. Simple classification variance analysis was performed on the data. The results indicated that the seeds had ovate to round shape; while the embryo was developed, of folded type. The fresh mass varied between 1,30 and 1,46 mg, and most of the seed resources were aimed at reserve formation (between 62,3 and 68,1 %). The moisture content varied between 11,5 and 13,2 %, and the desiccation tolerance index was lower than 0,5; value which is in correspondence with that of orthodox seeds. It is concluded that the information obtained on the biology of the *M. alba* seed is highly useful for the conservation of the germplasm bank of this species and for its sexual propagation.

Keywords: plant embryos, moisture, seeds.

Introduction

Since the 90's of last century, mulberry (*Morus alba* L. *Moraceae*) is studied in Cuba for sustainable forage production, due to its high acceptability by animals, and at present it is researched for its outstanding medicinal and silk industry potential (Martín *et al*., 2014). For such purpose, the country has a germplasm of 20 varieties preserved at the Pastures and Forages Research Station Indio Hatuey (EEPFIH), which were introduced from Costa Rica, Ethiopia, Brazil, South Korea, China and Spain (Martín *et al*., 2014).

This germplasm has been characterized, with the aim of evaluating its growth and development under the soil and climate conditions of different zones of Cuba and for its inclusion in the sustainable technologies which contribute to biomass production and the obtainment of bioproducts of interest for human, animal and plant health (Martín *et al*., 2014). However, if the mulberry crop is to be intensively and effectively exploited in the country, its seeds, as well as the germination mechanisms, should be accurately characterized.

The sexual reproduction of this species through seeds is essential to maintain genetic diversity. The knowledge of their biology is an important tool in order to be successful in the establishment of the seedlings in agricultural and silvopastoral systems, preserve a viable seed bank, make breeding programs and face climate change (Jiménez-Alfaro *et al*., 2016).

Among the seed traits the morphophysiological ones stand out, because they can be rapidly determined and have an important predictive value concerning plant adaptation (Sánchez *et al*., 2015). In this sense, Baskin and Baskin (2007; 2014) acknowledge that the internal structure of seeds, particularly embryo morphology, is a valuable piece of information for the classification of seed dormancy. Meanwhile, Jiménez-Alfaro *et al*. (2016) also state that seed size and mass are vitally important traits in the life cycle of a plant, because they have implications in the dispersal, establishment and survival mechanisms of the species. In addition, the hydration degree of disseminules plays a fundamental role in their longevity and germination performance.

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Nevertheless, other seed characteristics could also show the responses of the species to the environment; for example, the physical defense structures (testa/endocarp) and the nutrient content in the seed reserves –embryo/endosperm– (Daws *et al*., 2006; Montejo *et al*., 2015). For such reasons, the objective of this study was to characterize the different morphophysiological traits of the fresh seeds of five mulberry varieties which were harvested at the EEPFIH.

Materials and Methods

Plant material. The evaluated fresh *M. alba* seeds were from the genetic resource bank of the EEPFIH. As treatments seeds of five varieties were used: cubana, tigreada, universidad, universidad mejorada and yu-62, which were harvested in March, 2014. A completely randomized design was used for the trials, and they were performed immediately after collection, in the seed laboratory of the Institute of Ecology and Systematics –Havana, Cuba.

Seed characterization. The seed shape was described according to Niembro (1988), and the characteristics of the testa surface were detailed in agreement with the report by Stearn (1992). The description of the embryo type was carried out based on morphology (shape) and its degree of development (size), through the embryo length-seed length (S) relation, according to the classification criterion proposed by Baskin and Baskin (2007). For such purpose a sample of 30 seeds per variety was used, from which the embryos were extracted with a surgical scalpel. Afterwards, they were examined in a stereoscopic microscope equipped with micrometer, to measure the embryo length (mm). It was considered that the seed showed non-developed embryo (in terms of size) when it was small, but with differentiated organs, and the relation between the embryo size with regards to the seed (E-S) was lower than 0,5 mm. On the other hand, a seed was considered to have fully developed embryo when it occupied more than 50 % of the seed cavity (E-S > 0,5 mm) or the whole seed cavity (Baskin and Baskin, 2007).

Afterwards, from each variety a sample of 100 seeds was randomly taken, in which the seed dimensions were determined (length, width and diameter) with a caliper (Mitutoyo, of 0,02 mm accuracy). With these values the variance index of the seed dimensions was calculated, according to the method proposed by Thompson *et al*. (1993). Before the calculation of variance, each seed size value was divided by the length value, for the latter to be equal to the unit. Thus, in a spherical seed the variance is 0; while in an elongated or flattened one, the variance can be up to 0,33.

The other seed variables studied were: total fresh mass (mg), total dry mass (mg), initial moisture content (%) and dry mass of the reserves (embryo-endosperm, mg). The fresh mass was determined by individually weighing the seeds on a scale (Sartorius, with 10^{-4} g accuracy). The dry mass and moisture content were obtained from drying the seeds during 17 h, in a stove at 103 ± 2 ºC, according to the regulations of the International Seed Testing Association (ISTA, 2007). To calculate the fraction (or allocation) of the seed dry mass aimed at the seed reserves the value of this seed component was divided by the total seed dry mass (Sánchez *et al*., 2009), and the resulting values were multiplied by 100 to facilitate data interpretation.

The desiccation sensitivity probability index, *P* (D-S) was also determined, based on biometric data of the seeds, according to the formula proposed by Daws *et al*. (2006):

$$P\ (D\text{-}S) = \frac{e^{3,269-9,974a+2,156b}}{1+e^{3,269-9,974a+2,156b}}$$

where *a* represented the seed mass fraction aimed at the seed coats (MSC) and *b* is the $\log_{10}$ of the total seed dry mass. Therefore: if *P* (D-S) > 0,5 it is probable that the seeds are desiccation sensitive; if *P* (D-S) < 0,5 the seeds are likely to be desiccation tolerant; while if *P* (D-S) = 0,5 the seeds have the same probability to be desiccation sensitive or tolerant.

Statistical analysis. All the quantitative data were processed through a simple classification variance analysis, and in the case of the data expressed in percentage (moisture content and allocation to reserves) they were transformed with the arcsine of the square root of the proportion. For such purpose the program InfoStat v. 2015 (Di Rienzo *et al*., 2015) was used, taking into consideration that the fixed significance level was $p \leq 0,05$.

Results and Discussion

The *M. alba* seeds which were subject to analysis showed ovate to round shape; their color was light brown, and the testa surface, granulated to colliculate. Inside they showed a small layer of endosperm which completely surrounded the embryo, located in the central axis of the disseminule and whose organs were differentiated; which allows to state that it corresponds to a folded embryo (fig. 1),

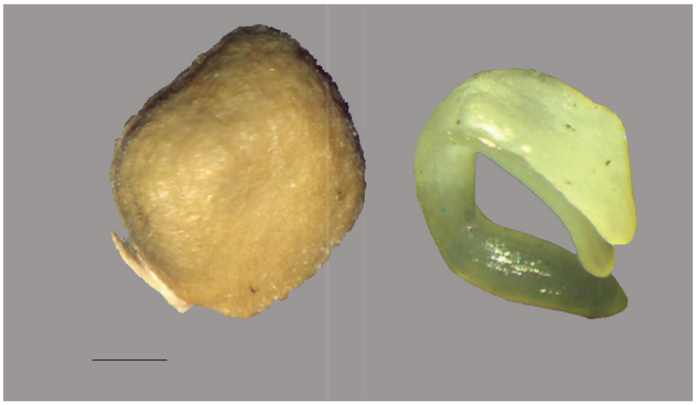

Figure 1. *Morus alba* seed and embryo (the scale bar equals 0,5 mm).

according to the characterization made by Baskin and Baskin (2007). This, in turn coincides with the characterization made by Baskin and Baskin (2014), for the Moraceae family. The embryonic axis was continuous, and the cotyledons, incumbent. The embryo size with regards to the inside of the seed cavity (E-S) was 50 % higher; thus, it is considered a developed embryo. This indicates that the dormancy classes the species can present, once the fruit is dispersed by the mother plant, are the physical, the physiological one, or combinations of these classes (Baskin and Baskin, 2014). In fact, for the fresh and aged *M. alba* seeds it has been reported that physical and physiological dormancy can exist (Barbour *et al*., 2008), although they can be non-dormant (Permán *et al*., 2013).

The length, width and diameter values of the *M. alba* seeds did not show significant differences among the varieties (table 1). The seed length varied in a range from 2,15 to 1,77 mm, and the seeds were thicker than wide. As the seed length was lower than 5 mm, this places the species in the smallest seed size category (class A), proposed by Hladik and Miquel (1990) for tree species. On the other hand, the variance of the dimensions showed that the seeds of all the varieties tended to be spherical, or ovate to round, as commented above.

The total (fresh and dry) seed mass, moisture content, biomass allocation to the seed reserves and desiccation sensitivity index did not show significant differences among the varieties (table 2). The average value of the fresh mass of the varieties was 1,41 mg, which corresponds to the second category of seed size (1,0-9,9 mg) proposed by Montejo *et al*. (2015) for tree species.

The initial moisture content varied between 11,5 and 13,2 %, with an average of 12,3 %; these moisture percentages were adjusted to the ones established for species with orthodox or desiccation tolerant seeds during storage (Dickie and Pritchard, 2002). In fact, the orthodox behavior of *M. alba* seeds is known (Permán *et al*., 2013; Royal Botanic Garden, 2015).

The values for the desiccation sensitivity index (< 0,5) also indicated that the seeds of the five varieties could be tolerant to desiccation. According to Permán *et al*. (2013), mulberry seeds can be maintained viable during two to three years under common environmental conditions. Likewise, it has been reported that in the Moraceae family more than 50 % of the species show orthodox or desiccation tolerant seeds (Dickie and Pritchard, 2002).

The seeds of the five varieties allocated more than 60 % of the total dry mass to the formation of

Table 1. Average values of the seed dimensions in the *M. alba* varieties.

Variety	Length (mm)	Width (mm)	Diameter (mm)	Variance of the dimensions
Cubana	2,06 (0,02)	0,96 (0,03)	1,63 (0,02)	0,07 (0,01)
Tigreada	2,07 (0,02)	0,92 (0,01)	1,58 (0,02)	0,07 (0,009)
Universidad	1,77 (0,02)	1,10 (0,02)	1,48 (0,02)	0,03 (0,01)
Universidad mejorada	1,83 (0,05)	1,03 (0,05)	1,46 (0,04)	0,04 (0,008)
Yu-62	2,15 (0,02)	1,00 (0,03)	1,65 (0,03)	0,07 (0,01)

( ): SE

Table 2. Average values of the morphophysiological traits of the seeds in the *M. alba* varieties

Variety	Fresh mass (mg)	Dry mass (mg)	Moisture content (%)	Allocation to reserves (%)	Sensitivity to desiccation
Cubana	1,46 (0,05)	1,28 (0,05)	12,3 (2,1)	68,1 (3,3)	0,0011 (1,6E-07)
Tigreada	1,29 (0,03)	1,15 (0,03)	11,5 (2,5)	64,8 (2,2)	0.0012 (1,6E-07)
Universidad	1,43 (0,07)	1,24 (0,06)	13,2 (1,9)	63,9 (2,8)	0.0010 (1,6E-07)
Universidad mejorada	1,40 (0,08)	1,22 (0,07)	12,8 (1,6)	67,8 (2,3)	0,0012 (1,6E-07)
Yu-62	1,48 (0,04)	1,21 (0,04)	11,9 (1,9)	62,3 (2,3)	0.0012 (1,6E-07)

( ): SE The percentage data are in correspondence with the original ones.

nutritional reserves (embryo/endosperm), as occurs in other species of very small seeds (Sánchez *et al*. (2009). This could guarantee that the seedlings have a certain amount of resources to grow during the first stages of their growth in nursery, phenomenon recorded in seeds from *Talipariti elatum* (Sw.) and *Ceiba pentandra* (L.) (Sánchez *et al.,* 2009). In addition, a considerable quantity of resources in the seed reserves could be an advantage for the beginning of growth on nutrient-poor soils (Sánchez *et al*., 2015).

Some of these traits have been identified in seeds from neotropical pioneer species, which grow in tropical evergreen and semideciduous forests, and also in *M. alba* seeds which grow in cultivated systems and wildly in semiarid Mediterranean zones (Permán *et al*., 2013).

Conclusions

The *M. alba* seeds showed a developed folded embryo, which occupied more than 50 % of the inside of the seed cavity, characteristic which showed that they did not show morphological or morphophysiological dormancy; but they could exhibit physiological dormancy, as occurs in other species of the Moraceae family. On the other hand, the seed mass values, moisture content and desiccation sensitivity index were in the range reported for the species with orthodox or desiccation tolerant seeds during the storage. For such reason, the information that was obtained about the biology of the *M. alba* seed is highly useful for the conservation of the germplasm bank of this species and for its sexual propagation.

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Bibliographic references

Barbour, J. R.; Read, R. A. & Barnes, R. L. *Morus* L. In: F. T. Bonner and R. P. Karrfalt, eds. *The woody plant seed manual*. 727. Washington: United States Department of Agriculture. Agriculture Handbook 727. p. 728-732, 2008.

Baskin, Carol C. & Baskin, J. M. *Seeds: ecology, biogeography and evolution of dormancy and germination*. 2 ed. San Diego, USA: Academic Press, 2014.

Baskin, J. M. & Baskin, Carol C. A revision of Martin's seeds classification system, with particular reference to this dwarf-seed type. *Seed Sci. Res*. 17 (1):11-20, 2007.

Daws, M. I.; Garwood, Nancy C. & Pritchard, H. W. Prediction of desiccation sensitivity in seeds of woody species: a probabilistic model based on two seed traits and 104 Species. *Ann. Bot*. 97 (4):667-674, 2006.

Di Rienzo, J. A.; Casanoves, F.; Balzarini, M.; González, L.; Tablada, M. & Robledo, C. W. *Manual del usuario. InfoStat*. Argentina: Grupo Infostat, Facultad de Ciencias Agrarias, Universidad Nacional de Córdoba. http://www.infostat.com.ar/index.php?mod=page&id=46. [13/10/2016], 2015.

Dickie, J. B. & Pritchard, H. W. Systematic and evolutionary aspects of desiccation tolerance in seeds. In: M. Black and H. W. Pritchard, eds. *Desiccation and survival in plants: drying without dying*. CAB International: Wallingford, The Netherlands. p. 239-259, 2002.

Hladik, A. & Miquel, S. Seedling types and plant establishments in an African rain forest. In: K. S. Bawa and M. Hardley, eds. *Reproductive ecology of tropical forest plants*. Paris: MAB, Parthenon Publishing Group. p. 261-276, 1990.

ISTA. *International rules for seed testing*. Bassersdorf, Switzerland: International Seed Testing Association, 2007.

Jiménez-Alfaro, B.; Silveira, F. A. O.; Fidelis, Alessandra; Poschlod, P. & Commander, Lucy E. Seed germination traits can contribute better to plant community ecology. *J. Veg. Sci*. 27 (3):637-645, 2016.

Martín, G. J.; Pentón, Gertrudis; Noda, Yolai; Contino, Y.; Díaz, Maykelis; Ojeda, F. *et al*. Comportamiento de la morera (*Morus alba* L.) y su impacto en la producción animal y la crianza de gusanos de seda en Cuba. *Rev. cubana Cienc. agríc*. 48 (1):73-78, 2014.

Montejo, Laura; Sánchez, J. A.; Muñoz, Bárbara & Gamboa, A. Caracterización de semillas de un bosque siempreverde tropical del oeste de Cuba. Correlaciones ecológicas entre rasgos. *Bosque (Valdivia)*. 36 (2):211-222, 2015.

Niembro-Roca, A. *Semillas de árboles y arbustos: ontogenia y estructura*. México, D. F.: Editorial Limusa, 1988.

Permán, J.; Navarro, R.; Nicolás, J. L.; Prada, María & Serrada, R., Coords. *Producción y manejo de semillas y plantas forestales*. Madrid: Organismo Autónomo Parques Nacionales, Ministerio de Agricultura, Alimentación y Medio Ambiente, 2013.

Royal Botanic Garden. *Seed Information Database (SID).* Version 7.1. Kew, United Kingdom: Royal Botanic Garden. http://data.kew.org/sid/. [19/04/2017], 2015.

Sánchez, J. A.; Montejo, Laura & Pernús, Mayté. Germinación de nuestras semillas: factor de éxito en la restauración ecológica. En: L. Menéndez, M. Arellano and P. M. Alcolado, eds. *¿Tendremos desarrollo socioeconómico sin conservación de la biodiversidad? Experiencias del Proyecto Sabana-Camagüey en paisajes productivos*. La Habana: Editorial AMA. p. 130-145, 2015.

Sánchez, J. A.; Muñoz, Bárbara & Montejo, Laura. Rasgos de semillas de árboles en un bosque siempreverde tropical de la Sierra del Rosario, Cuba. *Pastos y Forrajes*. 32 (2):141-164, 2009.

Stearn, W. T. *Botanical latin. History, grammar, syntax, terminology and vocabulary*. 4 th ed. United Kingdom: David & Charles, 1992.

Thompson, K.; Band, S. R. & Hodgson, J. G. Seed size and shape predict persistence in soil. *Funct. Ecol*. 7 (2):236-241, 1993.

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