Germination Capacity of *Annona deceptrix* (Westra) H. Rainer (Annonaceae) an Endemic and Endangered Species in Manabí, Ecuador

José Pico-Mendoza, Miryan Pinoargote, Luis Madrid, Juan Flor, Janner Álava, Gema Sancán, Carrasco Basilio, Ricardo Limongi, Geover Peña, Karla Quiroz

**ABSTRACT**

*Annona deceptrix* is an endemic and endangered tree from Ecuador according to the IUCN Red List of Endangered Species™. Its endangered status has been related with anthropogenic activities and some intrinsic characteristics of this species such as a low germination capacity of its seeds under natural conditions which is a serious limitation to obtain plants to establish conservation and breeding strategies. The objective of this study was to determine the seed germination capacity of *A. deceptrix* under different conditions and pre-germination treatments as a decisive factor in the survival of the species in ecosystems. The six pre-germination treatments were seed mechanical scarification (fine sandpaper), seed imbibitions in gibberellic acid solutions with three different concentrations (600, 700, 800 ppm) for 24 hours, imbibition of seeds in distilled water for 48 hours and direct sowing (control). For each treatment was sown twenty seeds under greenhouse conditions and germination chamber. The variables evaluated were: total number of germinated seeds, germination capacity, maximum germination value and germination energy. Mechanical scarification was the best treatment with 100% germinated seed and showed to be significantly different than the other treatment. In this regards, the rest of the treatment the germination ranged between 35% to 55% and did not show statistical differences each other. In conclusion, the seeds of *A. deceptrix* have a low germination capacity in natural conditions and then they need a pre-germination process such as mechanical-scarification to guarantees for their performance in the ecosystems.

**Key words:** *Annona deceptrix*, Endangered, Endemic plant, Pre-germination treatments, Scarification, Seed germination.

**INTRODUCTION**

The Annonaceae family is the largest of the Magnoliidae class (Lora et al., 2018) and is made up of trees, shrubs and rarely lianas (Maas, 2009). Worldwide around 2,500 species have been registered, but only twenty-three are cultivated (Khoshbakht and Hammer, 2008). *Annona* (now includes *Rollinia*), followed by *Asimina*, are the most agronomically interesting genus (George and Nissen 2003; Lora et al., 2018). Among then species like *Annona cherimola*, *A. squamosa*, *A. muricata*, *A. cherimola × A. squamosa* (hybrid) and *Asimina triloba* (Lora et al., 2018) are used for fresh consumption of their fruits. In addition *Annona* spp. have a high content of chemical compounds with biological activity and anticancer, analgesic and cytotoxic properties (Rabelo et al. 2016). In Ecuador, sixty-three species of *Annonas* are listed as useful (De la Torre, 2008) and about nineteen are endemic with some degree of threat (León Yánez et al., 2011). Within this group, there are some species such as *Annona manabiensis* Saff, ex R.E. Fr, *Annona conica*, Ruiz and Pav. ex G. Don and *Annona deceptrix* (Westra) H. Rainer with great potential to agricultural use. *A. deceptrix* is a tree commonly known as “chirimoya de monte or anonilla”, it is distributed in coastal wet and deciduous forests and is found at an elevation range of 0 to 850 m.a.s.l. (Muriel and Pitman, 2003). Unfortunately, it has been classified as an endangered species by the Red List of Threatened Species of IUCN and its vulnerability is due to anthropogenic activities (deforestation, erosion and land-use change) (León Yánez et al., 2011). There are also other threatening factors, such as borers that drill the fruit.
and feed on the endosperm, as in other commercials Annonas spp. (Hernández-Fuentes et al., 2017) and seed dormancy that is present in the Annonaceae family (Da Silva et al., 2007), thus, low seed germination capacity in natural conditions could be a problem in the survival of this species. The weight and morphology of the seed of A. deceptrix is not of greater relevance, how can it be in Lupinus albus (Hasan et al., 2018). In this case, the quality of the seed will depend on the appropriate moment of the harvest of fruits, as happens in other species as Capsicum annum (Tetteh et al., 2019).

There are some types of seed dormancy reported for Annonas spp. like morphological dormancy (small or underdeveloped embryos), physiological dormancy (physiological mechanisms that inhibit germination) and morphophysiological dormancy (small or underdeveloped embryos with physiological dormancy) (Baskin and Baskin, 2005). Annonas spp. have no physical dormancy because they do not have physical impediments to allow water into the seed and its coat is not impermeable (Lobo et al., 2007). It is well known that Annona spp. seeds need some help to germinate, so, some strategies have been developed to break seed dormancy. For example, Lobo et al. (2007) evaluated hot and cold stratification and the application of gibberellic acid to break the morphophysiological dormancy in A. cherimola and A. muricata. Martínez et al. (2016) obtained positive results by applying gibberellic acid to reduce morphophysiological dormancy in A. squamosa seeds, by same as Da Silva et al. (2007) in seeds of A. crassiflora and Carvalho et al. (2018) in A. cherimola Mill. x A. squamosa L. On the other hand, Ledo and Cabanelas (1997) found good results with mechanical scarification in seeds of A. muricata, as well as Adeniji et al. (2014) with A. squamosa to break non-deep physiological dormancy (Baskin and Baskin, 2004). Other studies showed a combination of strategies to reduce seed dormancy with good results such as Stenzel et al. (2005) with mechanical scarification with sandpaper + gibberellic acid (GA) in a concentration of 50 and 100 ppm and Lobo et al. (2007) with hot wet stratification + 800 ppm of GA, Annona plants can present one or several bottlenecks in their reproductive processes, which endangers the conservation of the species over time (Marbaniang et al., 2018). Therefore, the objective of this study was to determine the seed germination capacity of A. deceptrix under different conditions (greenhouse and germination chamber) and seed pre-germination treatments as a decisive factor in the survival of the species in ecosystems.

MATERIALS AND METHODS

Terms and definitions

Seeds germination was registered when the hypocotyl emerged on the soil surface in the greenhouse and when the radicle appeared in the germination chamber. The onset of germination (OG) indicates the time elapsed from the sowing of the seeds to the germination of 5% of the sown seeds. Germination percentage (GP) (ISTA, 2004), also known as germination power or germination capacity, quantifies the germination of seeds and the development of normal seedlings after planting. Maximum germination value (MGV) is the maximum ratio between the accumulated germination percentage and the number of days the test lasted. Germination energy (GE) is how fast the seeds germinate and develop a normal seedling.

Study location and seed characteristics

This study was carried out in greenhouse conditions, with averages of 70% humidity and 20°C temperature and in a germination chamber at 20°C with 65% humidity in the Facultad Ingeniería Agronómica of the Universidad Técnica de Manabí. Seeds were obtained from fruits of an individual of A. deceptrix located in the Cerro Pata de Pájaro forest in Pedernales, Manabí (Fig 1A). Fruition of this species is not very frequent, just once a year. Fruits have rarely been found in other seasons and normally they are pierced by insects (Fig 1B and C). The seeds were got from ripe fruits (Fig 1D), dried at room temperature and stored in paper bags.

Treatments

Germination tests were carried out in two conditions, first inside the greenhouse, simulating the natural conditions of the species and the second in the germination chamber. Six pre-germination treatments were tested (Table 1), each treatment with five seeds and four repetitions. The seeds were placed in plastic trays with a substrate (peat moss).
and irrigated daily, while in the germination chamber they were placed on paper towel moistened with sterile distilled water in Petri dishes. This species does not have many fruits, consequently the quantity of healthy seeds by fruits is limited. Sometimes it has been observed that this species can have fruits twice a year, but it is a function of the conditions where it is found.

Variables

Despite not carrying out viability tests of the embryos with tetrazole as suggested by the International Seed Testing Association (ISTA) (ISTA 2004), its viability could be determined by determining the percentage of seed germination (ISTA, 1999).

The evaluation was carried out every three days, recording the number of seeds germinated up to 40 days after sowing (DAS). Onset of germination (OG), germination percentage (GP), maximum germination value (MGV) and germination energy (GE) were determined.

\[
\text{GP (\%)} = \left(\frac{\text{SG}}{\text{SS}}\right) \times 100
\]

\[
\text{MGV} = \frac{\text{GP}}{\text{STG}}
\]

\[
\text{GE (\%)} = \left(\frac{\text{MSG}}{\text{SS}}\right) \times 100
\]

Where,

GP = germination percentage; SG = number of seeds germinate; SS = number of seeds sown; MGV = maximum germination value; STG = start of germination in days; GE = germination energy; MSG = most seeds germinate in a day.

These parameters refer to seed germination, they have been specified in different studies and in different species, such as in Kabuli chickpea, following the ISTA protocols (Gowda et al., 2018).

Statistical analysis

The tests of normality and homogeneity of the residues were carried out, through a Shapiro-Wilk test and inspection of the graphics of residues and predicted values. For the statistical analysis of the variables, an analysis of variance (ANOVA) was performed and mean comparisons with Tukey’s HSD test (p ≤ 0.05) through the InfoStat software (Balzarini et al., 2008).

RESULTS AND DISCUSSION

Currently the conservation of endangered species is becoming more important due to the accelerated degree of ecosystem deterioration. There are collections of these species through seed banks where they germination capacity and viability of the seed have been tested as part of the conservation process by knowing the biology of these species (Godefroid et al., 2010).

Scarcification

There are several ways that the seeds can germinate, where scarification or stratification treatments are highlighted to accelerate and break the dormancy of the seeds. Through scarification it has been possible to make way for embryos to contact air and water and to germinate. The results in this work show that the mechanical scarification in the greenhouse was the best treatment form germination of seed, starting at 27 DAS. However, scarified seeds in germination chamber, obtained only the 50% of the germinated seeds (p<0.05) (Fig 2). Germination of seeds with scarification was higher than other treatments (p = 0.02). Therefore it would be deduced that A. deceptrix has limitations for its reproduction and hence to secure their presence in the ecosystems. There are other factors that can restrict germination, such as heat and the amount of light, factors that affect other threatened species such as Asterolasia buxifolia (Collette and Ooi, 2017).

Our results resemble those of Patel et al. (2019) who obtained the maximum germination of seeds of A. squamosa when it was scarified and did not undergo any chemical treatment. Presumably because seed scarification reduces the seed coat size and facilitates its rupture. For example, A. squamosa seed germination occurs in two separate and consecutive phases: 1) the rupture of the seed coat and 2) the rupture of the endosperm (Martinez-Maldonado et al., 2013). Also, scarification is related to reducing the resistance of the seed coat so that the radicle can exit faster (Baskin and Baskin 2004). As a result, A. deceptrix scarified seed germinated faster than the others did. Our findings are similar to the high germination percentage in seeds of A. muricata (84% GP) (Lcedo and Cabanelas, 1997) and A. squamosa (80% GP) (Adeniji et al., 2014) with mechanical scarification.

However, in species such as A. muricata, mechanical scarification with sandpaper or stones was not positive for germination (Dada et al., 2019). In this case, chemical scarification with 50% sulfuric acid for 5 minutes was an efficient treatment to break dormancy in A. muricata seeds,

### Table 1: Pre-germination treatments applied in seeds of A. deceptrix under greenhouse conditions and germination chamber in Santa Ana canton, province of Manabi, Ecuador.

| Treatments | Pre-germination methods | Description |
|------------|-------------------------|-------------|
| T1         | Mechanical scarification | Minimum wear of seed coat with sandpaper |
| T2         | Soaking of seeds in 600 ppm GA<sub>3</sub> | Imbibition of seeds for 24 hours |
| T3         | Soaking of seeds in 700 ppm GA<sub>3</sub> | Imbibition of seeds for 24 hours |
| T4         | Soaking of seeds in 800 ppm GA<sub>3</sub> | Imbibition of seeds for 24 hours |
| T5         | Water                   | Imbibition of seeds for 48 hours |
| T6         | Control                 | Direct sowing |

Note: GA<sub>3</sub> = gibberellic acid.
presenting 60% germination and soak the seeds in coconut water for 15 minutes, represented with 39% germination (Dada et al., 2019).

Maximum germination value (2.5 germinated seeds per day) and germination energy (45%) were higher in the scarified seeds in greenhouse conditions (Table 2). Pre-germination treatments in germination chamber shown values close to zero, the germination capacity of seeds were less than 40% (Fig 3).

**Imbibition in gibberellic acid and water**

In this study gibberellic acid (GA) had not effect on seed germination; moreover, treatments with doses higher than 700 ppm of GA had lower GP than the control treatment (Fig 2).

In contrast to our results seed germination in other Annona species have shown favorable results with GA. For instance, good results were obtained using low concentrations in vitro conditions with 3 mg GA in A. reticulata and A. cherimola (86 and 82% GP respectively) (Kudikala et al., 2018; Padilla and Encina, 2003), 50 and 100 mg GA + mechanical scarification in Annona squamosa and Annona cherimola Mill. × Annona squamosa L. (75 and 44% GP respectively) (Stenzel et al., 2005); 600 mg GA in Annona squamosa L. (95% GP) up to 800 mg GA in Annona cherimola L. and Annona muricata L. (83 and 62% GP respectively) (Lobo et al., 2007), these results are the opposite of the result of this study. The lowest germination capacity (35%) was observed in seeds in T4 (800 mg L⁻¹ of GA) under greenhouse conditions and 25% in treatments T2, T3, T4 (600,700,800 mg L⁻¹ of GA) germination chamber conditions.

These results show that concentrations above 600 ppm can cause germination problems and the best results in different species of Annona are in low concentrations of gibberellic acids, a condition that is similar to A. squamosa species (Jain et al., 2017).

In this case, many times the embryo in the seeds is covered by an impermeable membrane and the micropyle is the only entrance through which water enters, as happens in A. cherimola (De Smet et al., 1999).

Seed imbibitions in water for 48 hours showed the lowest germination capacity (20%) in the germination chamber, Ledo and Cabanelas (1997) also found the lowest result (7%) in the imbibition of A. muricata seeds in water for 24 hours, half our study.

**Table 2:** Germination tests on seeds of *A. deceptrix* under greenhouse conditions (GH) and germination chamber (CH) under six pre-germination treatments in Santa Ana, Manabí, Ecuador.

| T     | SG   | GP (%) | MGV  | GE (%) |
|-------|------|--------|------|--------|
|       | GH   | CH     | GH   | CH     | GH   | CH   |
| T1    | 5±0.0a | 2±0.8b | 100±0.0a | 40±11b | 2.5±0.0a | 1±0.4b | 45±19a | 15±10b |
| T2    | 2.7±0.5b | 1.2±0.5b | 55±10b | 25±10b | 1.4±0.2b | 0.6±0.2b | 10±6b | 0±0b |
| T3    | 2±0.8b | 1.2±0.5b | 40±16b | 25±10b | 1±0.4b | 0.6±0.2b | 0±0b | 0±0b |
| T4    | 1.7±0.5b | 1.2±0.5b | 35±10b | 25±10b | 0.9±0.2b | 0.6±0.2b | 5±3b | 0±0b |
| T5    | 2.5±1.9b | 1±0.8b | 50±30b | 20±16b | 1.3±0.9b | 0.5±0.4b | 5±3b | 0±0b |
| T6    | 2.5±0.5b | 2±0.8b | 50±11b | 40±16b | 1.3±0.2b | 1.0±0.4b | 0±0b | 0±0b |

Notes: SG= total number of seeds germinate; GP= germination percentage; MGV= maximum germination value; GE= germination energy; GA= gibberellic acid; T= treatments; T1= Mechanical scarification; T2= Soaking of seeds in 600 ppm GA (24 hours); T3= Soaking of seeds in 700 ppm GA (24 hours); T4= Soaking of seeds in 800 ppm GA (24 hours); T5= water (48 hours); T6= control.

Different letters within each column indicate significant differences between treatments (Tukey, p <0.05). Means ± standard deviation.

**Fig 2:** Seeds of *A. deceptrix* germinated in greenhouse conditions and germination chamber under six pre-germination treatments in Santa Ana, Manabí, Ecuador.

Notes: GA= gibberellic acid; T1= Mechanical scarification; T2= Soaking of seeds in 600 ppm GA (24 hours); T3= Soaking of seeds in 700 ppm GA (24 hours); T4= Soaking of seeds in 800 ppm GA (24 hours); T5= water (48 hours); T6= control.
Low germination of Annonas

Low germination of A. deceptrix in natural conditions coincides with the available literature of Annonas spp., e.g. seeds of A. cherimola Mill. X A. squamosa L. without pre-germination treatments had 1% GP (Stenzel et al., 2005) and A. reticulata 8.8% GP (Kudikala et al., 2018). These results showed that these species struggle to maintain a high population in natural conditions. The time it takes for the seeds to germinate is also another limitation and species such as A. crassiflora can take up to 150 days to germinate (Da Silva et al., 2007). In this study, the seeds with the lowest germination percentage (20%) were those imbibed in water for 48 hours in the germination chamber and the ones that took longer to germinate (34 DAS) were the control seeds in greenhouse conditions.

There are other factors that affect the germination of the Annona seed, such as the position inside of the fruit and the presence of a micropylar woody plug in the case of A. macroprophyllata (González-Esquinca et al., 2015).

Endangered species should receive special treatment, since there are no major basic studies about them. Currently, the references available related to Annonaceae family or genus, they are in commercial species, but there are no major reports in endangered species. Due to this, it becomes necessary to study on reproductive aspects of the species and determine the type of dormancy present in them, as in this case, Ribes echinellum, threatened species located in Southeastern US it has morphophysiological dormancy, which indicates that it needs hormonal treatments for germination (Negrón-Ortiz, 2018).

This study highlighted that germination capacity of A. deceptrix depends on some factor that breaks the dormancy of the seed and if in natural conditions it does not find any factor that promotes this process; the species permanence of this species in ecosystems is at risk. Likewise, this seed possess a high germination capacity but it needs to break seed dormancy in a short time. In addition, it has been observed that there are insects that perforate the ripe fruits and damage the embryo. This situation further increases the low seed germination rate by decreasing the number of viable seeds.

Our result confirms that A. deceptrix is a species that deserves special attention, as well as with other threatened species; A. deceptrix is forgotten and underutilized because it does not have an economic value, yet that is why in-situ and ex-situ conservation strategies (Kour et al., 2018) are vital until species domestication program could be achieved (Sakthivel et al., 2019).

CONCLUSION

This is the first study of the seed germination capacity of an endemic and endangered A. deceptrix from the Ecuadorian coast. A. deceptrix has limitations for its reproduction in natural conditions and hence to secure its presence in the ecosystems, the seeds need a pre-germination process and scarification promotes the higher germination capacity of the seeds. Although the literature varies greatly regarding the germination processes in Annonaceae, it has been shown that gibberellic acid effectively promotes germination, but it is not a factor that acts only in this process and the germination of the seed is conditioned to other factors of great importance like the maturity of the embryo, quality of the fruits, hardness of the cover, healthy seeds, as well as the humidity, type of soil and luminosity.

REFERENCES

Adeniji, I. T., Adio, A. F., Iroko, O. A., Kareem, A. A., Jegede, et al. (2014). Pre-Treatment of Seeds of Annona Squamosa (Sugar Apple) A Non Timber Forest Product. Scientific Research in Plant Science, 2(3): 50–52. https://doi.org/10.12691/plant-2-3-1.

Balzarini, M. G., Gonzalez, L., Tablada, M., Casanoves, F., Rienzo, J. A. Di and C.W. Robledo. (2008). Manual del Usuari. Editorial Brujas, Córdoba, Argentina. Editorial Brujas, Córdoba, Argentina.
Germination Capacity of *Annona depressa* (Westra) H. Rainer (Annonaceae) an Endemic and Endangered Species in Manabí, Ecuador

Baskin, C. C. and Baskin, J. M. (2005). Seed dormancy in trees of climax tropical vegetation types. Tropical Ecology. 46(1): 17–28.

Baskin, J. M. and Baskin, C. C. (2004). A classification system for seed dormancy. Seed Science Research. 14: 1–16. https://doi.org/10.1079/SSR2003150.

Carvalho, D. U. de, Cruz, M. A. da, Osipi, E. A. F., Cossa, C. A., Colombo, R. C., and Sorace, M. A. F. (2018). Plant growth regulators on atemoya seeds germination. Nucleus. 15(2): 457–462. https://doi.org/10.3738/1982.2278.2832.

Collette, J. C. and Ooi, M. K. J. (2017). Germination ecology of the endangered species Asterolasia buxifolia ( Rutaceae): Smoke response depends on season and light. Australian Journal of Botany. 65(3): 283–291. https://doi.org/10.1071/BT17025.

Da Silva, E. A. A., De Melo, D. L. B., Davide, A. C., De Bode, N., Abreu, G.B., Faria, J. M. R., and Hilhorst, H. W. M. (2007). Germination ecophysiology of *Annona crassifolia* seeds. Annuals of Botany. 99(5): 823–830. https://doi.org/10.1093/aob/mcm016.

Dada, C., Kayode, J., Arowosede, S. and Ayeni, M. (2019). Effect of scarification on breaking seed dormancy and germination enhancement in *Annona muricata* L. (Magnoliales: Annonaceae): World Scientific News. 126(April): 136-147.

De la Torre, L. (2008). *Enciclopedia de las plantas útiles del Ecuador*. Herbario QCA de la Escuela de Ciencias Biológicas de la Pontificia Universidad Católica del Ecuador. Retrieved from http://repositorio.educacionesuperior.gob.ec/handle/28000/3705.

De Smet, S., Damme, V. P. X., S. and Romero, J. (1999). Seed structure and germination of cherimoya. In: First International Symposium on Cherimoya, pp. 259–278.

George, A. P. and Nissen, R. J. J. (2003). Annonaceous fruits. In: Encyclopedia of Food Sciences and Nutrition, Academic Press, pp. 239–245.

Godefroid, S., van de Vyver, A. and Vanderborght, T. (1998). Germination capacity and viability of threatened species collections in seed banks. Biodiversity and Conservation. 19(5): 1365–1383. https://doi.org/10.1007/s10531-009-9767-3.

González-Esquínca, A. R., De-La-Cruz-Chacón, I., and Domínguez-Gutú, L. M. (2015). Dormancy and germination of *Annona macrophylla* (Annonaceae): The importance of the micropylar plug and seed position in the fruits. Botanical Sciences. 93(3): 509-515. https://doi.org/10.17129/botsci.166.

Gowda, B., Naik, A. K., Rakesh, Mathad, C., Ganiger, B. S., Lokesh, G. Y., Rekha. (2018). An improved method of seed germination testing in Kabuli chickpea. Indian Journal of Agricultural Research. 52(4): 456-459. https://doi.org/10.18805/IJARRe.4879.

Hasan, M. A., Al-Taweel, S. K., Hamza, J. H. and Jawad, W. M. (2018). Effect of seed weight on stem anatomical characters in white lupine (*Lupinus albus L.*) cultivars. Indian Journal of Animal Research. 52(6): 666–670. https://doi.org/10.18805/IJARRe.A-352.

Hernández-Fuentes, L. M., Bautista Martínez, N., Carrillo-Sánchez, J. L., Sánchez Arroyo, H., Urias-López, M. A. and Salas Alzúa, M. D. (2017). Control del barrenador de las semillas, bephratoilodes cubensis ashmead (hymenoptera: eurytomidae) en guánabana, annona muricata l. (annonales: annonaceae). Acta Zoológica Mexicana (N.S.), 24(1): 199–206. https://doi.org/10.21829/azm.2008.241631.

ISTA. (1999). International Rules for Seed Testing: Seed Science and Technology. International Seed Testing Association, Zurich, Switzerland, pp: 24.

ISTA. (2004). International rules for seed testing. Basserdorf, Switzerland, International Seed Testing Association Zurich. Jain, S., Sharma, T. R., Lal, N., Rangare, N. R. and Kumar, B. (2017). Effect of GA 3 and Growing Media on Seedling Vigour and Physiological Parameter of Custard Apple (*Annona squamosa* L.). International Journal of Chemical Studies. 6(8): 606–615.

Khoshbakht, K. and Hammer, K. (2008). Species Richness in Relation to the Presence of Crop Plants in. Journal of Agriculture and Rural Development in the Tropics and Subtropic. 109(2): 181–190.

Kour, S., Bakshi, P., Sharma, A., Wall, V. K., Jasrotia, A. and Kumari, S. (2018). Strategies on Conservation, Improvement and Utilization of Underutilized Fruit Crops. International Journal of Current Microbiology and Applied Sciences.7(03): 638-650. https://doi.org/10.20546/ijcmas.2018.703.075.

Kudikala, H., Ellendula, R., Nazhin, S., Sirikonda, A., Mood, K. and Allini, V. R. (2018). Effect of pre-treatment methods on *in vitro* seed germination of bulbuck’s heart (*Annona reticulata* L.). Asian Journal of Plant Sciences. 17(3): 142-149. https://doi.org/10.3923/ajps.2018.142.149.

Ledo, A. D. S. and Cabanelas, C. I. L. (1997). Superação de dormência de sementes de gra viola (*Annona muricata* L.). Rev Bras Fruticultura. 19(3): 397-400.

León Yáñez, S., Valencia, R., Pitman, N., Endara, L., Ulloa-Ulloa, C. and Navarrete, H. (2011). Libro Rojo de las Plantas Endémicas del Ecuador.

Lobo, M., Delgado, O., Cartagena, J. R., Fernández, E., and Medina, I. (2007). Categorización de la germinación y la latencia en semillas de chirimoya (*Annona cherimola* L.) y guanabana (*Annona muricata* L.), como apoyo a programas de conservación de germoplasma. Categorization of germination and dormancy of cherimoya (*Annona cherimola*) and guanabana (*Annona muricata*), as an aid to programs of germplasm conservation. Agronomía Colombiana. 25(2): 231-244.

Lora, J., Larranaga, N. and Hormaza, J. I. (2018). Genetics and Breeding of Fruit Crops in the Annonaceae Family: Anno*na* spp. and *Asimina* spp. In: Advances in Plant Breeding Strategies: Fruits, [J.M. Al-Khayri, S.M. Jain and D.V Johnson (Eds.)], Cham: Springer International Publishing. (pp. 651-672). https://doi.org/10.1007/978-3-319-91944-7_16.

Maas, P. J. M. (2009). Neotropical Annonaceae. In: Milliken, W., Kitlgard, B. and Baracat, A. (2009 onwards), Neotropikey - Interactive key and information resources for flowering plants of the Neotropics. Retrieved from http://www. kew.org/science/tnopamerica/neotropikey/familiesAnnonaceae.htm.

Marbaniang, E. J., Venugopal, N., Verma, S., Raina, R., Khajuria, A. and Gautam, K. (2018). Floral biology and embryological studies are important for conservation of threatened plants having reproductive bottlenecks:/ a case study of *Illicium griffithii* Hook. 1. and Thomson. Current Science. 114(3): 576–587. https://doi.org/10.18520/cs/v114/i03/576-587.

Martínez-Maldonado, F. E., Miranda-Lasprilla, D. and Magnitskiy, S. (2013). Sugar apple (*Annona squamosa* L., *Annonaceae*)
Germination Capacity of Annona deceptrix (Westra) H. Rainer (Annonaceae) an Endemic and Endangered Species in Manabí, Ecuador

Patel, P., Pathak, J. and Suthar, R. (2019). Effect of Various Physicochemical Treatments on Seed Germination of Annona squamosa L. Asian Journal of Applied Sciences. 12(3): 128–132. https://doi.org/10.3923/ajaps.2019.128.132.

Sakthivel, T., Senthil Kumar, R. and Bonath, S. (2019). Management and Conservation of Underutilized Fruits. In: Conservation and Utilization of Horticultural Genetic Resources, [P. E. Rajasekharan and V. R. Rao (Eds.)] Singapore: Springer Singapore. https://doi.org/10.1007/978-981-13-3669-0_13. pp. 409–424.

Patel, P., Pathak, J. and Suthar, R. (2019). Effect of Various Physicochemical Treatments on Seed Germination of Annona squamosa L. Asian Journal of Applied Sciences. 12(3): 128–132. https://doi.org/10.3923/ajaps.2019.128.132.

Rabelo, S.V., Quintans, J.D.S.S., Costa, E.V., Almeida, J.R.G.S. and Quintans Júnior, L. (2016). Annona Species (Annonaceae) Oils. In: Essential Oils in Food Preservation, Flavor and Safety, Academic Press. pp. 221–229.

Patel, P., Pathak, J. and Suthar, R. (2019). Effect of Various Physicochemical Treatments on Seed Germination of Annona squamosa L. Asian Journal of Applied Sciences. 12(3): 128–132. https://doi.org/10.3923/ajaps.2019.128.132.

Stenzel, N. M. C., Murata, I. M. and Neves, C. S. V. J. (2005). Superação da dormência em sementes de atemóia e fruta-do-conde. Revista Brasileira de Fruticultura. 25(2): 305–308. https://doi.org/10.1590/s0100-29452003000200031.

Tetteh, R., Aboagye, L. M., Darko, R. and Osafo, E. A. (2019). Physiological seed quality in relation to maturity stage in two pepper (Capsicum annuum L.) cultivars. Agricultural Reviews. 53(5): 604–608. https://doi.org/10.18805/ijare.a-419.