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MARCELO DO NASCIMENTO ARAUJO

PHYSIOLOGICAL ASPECTS OF GERMINATION AND STORAGE OF *Amburana cearensis* (Allemão) A.C.Sm. (Fabaceae) SEEDS

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Thesis presented to the post-graduate program in Plant Genetic Resources, of State University of Feira de Santana as a partial requirement to obtain the title of Doctor in Plant Genetic Resources.

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“A minha noiva Carla Araujo, com muito amor. ”

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"*A verdadeira motivação vem de realização, desenvolvimento pessoal, satisfação no trabalho e reconhecimento*."

Frederick Herzberg

ARAUJO, M.N. 2017. **Physiological aspects of germination and storage of *Amburana cearensis* (Allemão) A.C.Sm. (Fabaceae) seeds**. 78p. Thesis (Doctorate in Plant Genetic Resources) – State University of Feira de Santana (UEFS), Feira de Santana, BA, 2017.

Amburana cearensis (Allemão) A.C.Sm. is native tree of Brazil adapted at semi-arid habitats. It has ecological, commercial and medicinal importance. *A. cearensis* is listed in the red list of endangered species. It is threatened by habitat loss and exploitation for use in folk medicine. The bark is used, in the traditional medicine, to cure respiratory diseases while seeds are used to treat lung diseases. This work aims to study the physiological aspects of germination and storage of *Amburana cearensis* (Allemão) A.C.Sm. seeds. Four storage conditions were used and assessed during 27 months: airtight container in refrigerator; airtight container in laboratory, paper bags in laboratory and liquid nitrogen during 24 months. Germination test was performed at temperatures of 15, 20, 30, 35, 40 and 45 ºC with a photoperiod of 12 hours. Germination in salt solutions was used salt concentration of 100, 200, 300, 400 and 500 mM. *A. cearensis* seeds kept in refrigerated environment maintain the viability for at least two years. The ideal temperature in seed germination of *A. cearensis* is 38 ºC. Accessions differed in seed dry mass, in time until 50% imbibition (IM*t50*), and time until radicle protrusion (RP). The start of water uptake (TWU) was delayed by more than 4 d despite optimal contact between the seed surface and water, and this delay was stronger for smaller seeds and differed between accessions. Longer delay of imbibition was also correlated with higher optimum temperature for germination rate (T_o), and with longer time until radicle protrusion in water. The TWU, IM*t50*, and the RP differed between water and salt treatments for the accessions from the semi-arid habitat. These results suggest that it is not advisable to store *A. cearensis* seeds in laboratory environment without an airtight container and the delayed of the water uptake forms an adaptation to an environment with high temperature, low precipitation, and saline soils, most likely to spread the risk of completing germination at the start of the rainy season.

Keywords: Caatinga, Fabaceae, Leguminosae, Medicinal Plants, Storage, Umburana-de-cheiro.

ARAUJO, M.N. 2017. **Aspectos fisiológicos da germinação e armazenamento de sementes de *Amburana cearensis* (Allemão) A.C.Sm**. **(Fabaceae)** 78p. Tese (Doutorado em Recursos Genéticos Vegetais) – Universidade Estadual de Feira de Santana (UEFS), Feira de Santana, BA, 2017.

Amburana cearensis (Allemão) A.C.Sm. é uma árvore nativa do Brasil adaptada a habitats semiáridos. Tem importância ecológica, comercial e medicinal. *A. cearensis* está inserida na lista vermelha de espécies ameaçadas de extinção. É ameaçada por perda do habitat e exploração para o uso na medicina popular. A casca é utilizada, na medicina tradicional, para curar doenças respiratórias enquanto as sementes são usadas para tratar doenças pulmonares. Este trabalho tem como objetivo estudar os aspectos fisiológicos da germinação de sementes de *Amburana cearensis*. Foram utilizadas quatro condições de armazenamento e avaliados durante 27 meses: recipiente hermético em geladeira; recipiente hermético em laboratório; sacos de papel em laboratório e nitrogênio líquido. O teste de germinação foi realizado em temperaturas de 15, 20, 30, 35, 40 e 45 ºC com fotoperíodo de 12 horas. Para germinação em soluções salinas foi utilizada concentração de 100, 200, 300, 400 e 500 mM. As sementes de *A. cearensis* mantidas em ambiente refrigerado mantiveram a viabilidade durante pelo menos dois anos. A temperatura ideal na germinação de sementes de *A. cearensis* é de 38 ºC. Os acessos diferiram entre si na massa seca da semente, no tempo até 50% de imbibição (IM*t50*) e de protrusão da radícula (RP). O início da absorção de água (TWU) foi atrasado em mais de 4 d, apesar do ótimo contato entre a superfície da semente e a água, e este atraso foi mais forte para as sementes menores diferindo entre os acessos. O atraso maior da embebição também foi correlacionado com uma temperatura ótima mais alta para taxa de germinação (T_o), e com maior tempo até protrusão da radícula em água. O TWU, o IM*t50* e o RP diferiram entre tratamentos de água e sal para as acessos do habitat semiárido. Estes resultados sugerem que não é aconselhável armazenar sementes de *A. cearensis* em ambiente de laboratório sem recipiente hermético e o atraso da absorção de água forma uma adaptação a um ambiente com alta temperatura, baixa precipitação e solos salinos, muito provavelmente para espalhar o risco de completar a germinação no início da estação chuvosa.

Palavras-chave: Armazenamento, Caatinga, Fabaceae, Leguminosae, Plantas Medicinais, Umburana-de-cheiro.

SUMMARY

1.0 GENERAL INTRODUCTION

Caatinga biome is characterized by xerophytic vegetation, low rainfall around 500-700 mm per year. This type of plant formation has well defined characteristics: low trees and shrubs generally lose leaves in the dry season in addition to the vegetation in general aspect, spiny bush with a desert physiognomy. In addition to these severe climatic conditions, Caatinga is subject to strong and dry winds, which contribute to the landscape of drought during the dry season (ARAÚJO; SOUSA, 2011; LIMA, 1996 and SANTOS; ANDRADE, 1992).

Amburana cearensis (Allemão) A.C.Sm. popularly known as "umburana-de-cheiro" belongs to the Fabaceae. With great contribution to Caatinga biome *A. cearensis* commonly found in Northeastern Brazil from the Northeast to São Paulo in the South-west. It can grow not only in semi-arid environments but also shows good adaptation to rain forest. It has commercial importance of its various applications, widely used in carpentry, perfumery and pharmaceutical purposes. This is one of reasons that it is listed as an endangered species (HILTON-TAYLOR, 2000).

Seed quality is characterized by genetic, physiological and physical health and of fundamental importance in the production process of any plant species. For forest seeds, the quality is generally evaluated by the germination test and vigour, carried out under controlled conditions, to try simulating the natural environment occur environmental when differences occur that may affect the behaviour of seeds and seedlings (POPINIGIS, 1985).

Therefore, knowledge about the behaviour of seed germination and seedling of species as *A. cearensis* are of fundamental importance for studies related to seed conservation. Considering that storage period interferes on quality and quantity of seedlings obtained and, consequently, the production performance of the established population in the field. Maintaining the viability of the seeds by storing in controlled environmental conditions, it has been one of the most important lines of research for the large number of species of seeds (BATISTA, 2015).

In a scenario in which tree growth rates haves been decreasing in response to warming or drought stress in many forests around the world (ALLEN et al., 2010), phenomenon that is attributed to climate change-driven and drought events (WILLIAMS et al., 2013). Thus, the plant ecosystems may suffer negative influences and 18% of species will be endangered until 2050 (THOMAS et al., 2004).

Temperature and water are the most important environmental factors for seed germination (BEWLEY et al., 2013). The suitable temperature in the germination is related to better performance of cellular biochemical processes improving the speed and germination uniformity (CARVALHO; NAKAGAWA, 2012). When seeds have similar behaviour in variable temperatures and there is a great and uniform germination temperature. In general, the optimum germination temperature occurs when presents the maximum germination in the shortest time (DOUSSEAU et al., 2011).

Soil salinity and sodicity problems are common in arid and semi-arid areas, where precipitation is insufficient to leach the salts and sodium ions in excess out of the rhizosphere. The salt stress represents one of the most serious factors that limit growth and crop production, inducing morphological changes, structural and metabolic disorders in higher plants (AZEVEDO-NETO, 2000). Since this stress affect the time and the rate of seed germination, the height of the plant, the size of the branches and the growth of the leaves, so all plant anatomy and morphology (POLJAKOFF-MAYBER; GALÉ, 1975).

The uptake of water by seeds is triphasic standard. Phase I, imbibition, it is the result of matric potential and, therefore it is a physical process occurring independently of seed viability. Phase II called stationary, it occurs due to the balance between the osmotic potential and the potential pressure. Phase III, is characterized by the return of water absorption, resulting in the emission of primary root (BEWLEY et al., 2013). Some authors have studied this triphasic model germination in seeds of native species from Caatinga as *Bauhinia cheilantha* (Bong.) Steud. (Fabaceae), *Poincianella pyramidalis* (Tul.) L.P.Queiroz (Fabaceae), *Schinopsis brasiliensis* Engl. (Anacardiaceae) (DANTAS et al., 2007a; DANTAS et al., 2007b and SILVA et al., 2004).

During development, seed deterioration is inevitable and variable among species, batches of the same species and among units of the same batch. The probable sequence of deterioration involves degeneration of cell membranes, damage in energetic production mechanisms and biosynthesis, reduction in germination speed, storage reduction, desuniformity and retardation of growth and development of seedlings, increase in the sensitivity to environmental diversities, the reduction in seedling emergence in the field, increase occurrence of abnormal seedlings and death (DELOUCHE; BASKIN, 1973).

For *ex-situ* conservation is necessary to choose a strategy to ensure the survival of the species, making it necessary to initially know the germination behaviour of seeds over different periods of storage, ie, seed longevity and study which conditions that provide good longevity.

Current knowledge of seed storage techniques is limited to plants of agricultural interest, is not very well known about the requirements of the majority of the seeds of wild species (HEYWOOD, 1989).

There is a large amount of studies of storage of forest species, however, the knowledge is not as broad as in cultivated plants. With *Amburana cearensis* there are other works, but not to the same extent as current study (DANTAS et al., 2008; GUEDES et al., 2010a and LÚCIO, 2010). Considering the importance of *Amburana cearensis* on Caatinga biome and to study the behaviour and mechanisms of adaptation of native species from Caatinga under adverse conditions, this research attempt to test physiological aspects of germination and conservation in different storage conditions.

2.0 OBJECTIVES

2.1 General

Study the physiological aspects of germination and storage of *Amburana cearensis* (Allemão) A.C.Sm. seeds.

2.2 Specific

Evaluate the germination behaviour of *A. cearensis* seeds in different times and storage conditions.

Study the vigour of *A. cearensis* seeds in different times and storage conditions.

Obtain the values of optimum temperature under germinations responses in *A. cearensis* seeds.

Characterize the process of seed imbibition of 8 accessions of *A. cearensis* and analyze the behaviour of the seeds during water uptake.

Study responses of 8 accessions of *A. cearensis* seeds to salt stress.

3.0 LITERATURE REVIEW

3.1 The species

Two species belong to the genus *Amburana*: *A. cearensis* (Allemão) A.C.Sm. and *A. acreana* (Ducke) A.C.Sm. According to the new classifications (HAWKINS et al., 2017) *A. cearensis* belongs to the subfamily Papilionoideae.

Amburana cearensis is known under different popular names in its range: imburana-de-cheiro, umburana-de-cheiro, cerejeira, cumaru (Northeast Brazil), amburana, cumaru-das-caatingas (Southeast Brazil), roble criollo (Argentina), tumi (Bolivia) and trébol (Paraguay) (MELO et al., 2015; Figure 2C).

The *A. cearensis* is often confused with the species *Dipteryx odorata* (Aubl.) since the popular denomination cumaru of both species. The common name imburana causes similar mistakes in identification with *Commiphora leptophloeos* (Burseraceae), known commonly as imburana-de-espinho (MAIA, 2008 and PIO-CORRÊA, 1984).

3.1.1 Geographical distribution

Amburana cearensis occurs in Caatinga, Cerrado and Atlantic rainforest biomes (shrubby savannah) of Central and Central-West Brazilian regions but also in. A characteristic of this species is its adaptation to poor, calcareous soils (SILVA, 2003) and dry forest (RAMOS, 2004). Therefore, there are reports of its distribution in other South American countries: Northern Argentina, Southern Bolivia, Paraguay and Northeast of Peru (RAMOS, 2004).

In Brazil, *A. cearensis* has its largest distribution in the Caatinga and the centre of the Cerrado. The species also extends to the midwest and Southeast to form the largest part of the distribution of the species. The distribution in the west includes the states of Goiás, Minas Gerais, Mato Grosso do Sul, and to the south, to reach the State of the São Paulo and the Atlantic coast of state of Espírito Santo. The expansion to the south reaches its maximum at the Tropic of Capricorn in the most western sites. This species is also found in the Brazilian State of Acre, and the border of Peru, Bolivia and Paraguay (CORREA, 1984 and LORENZI, 2008; Figure 1).

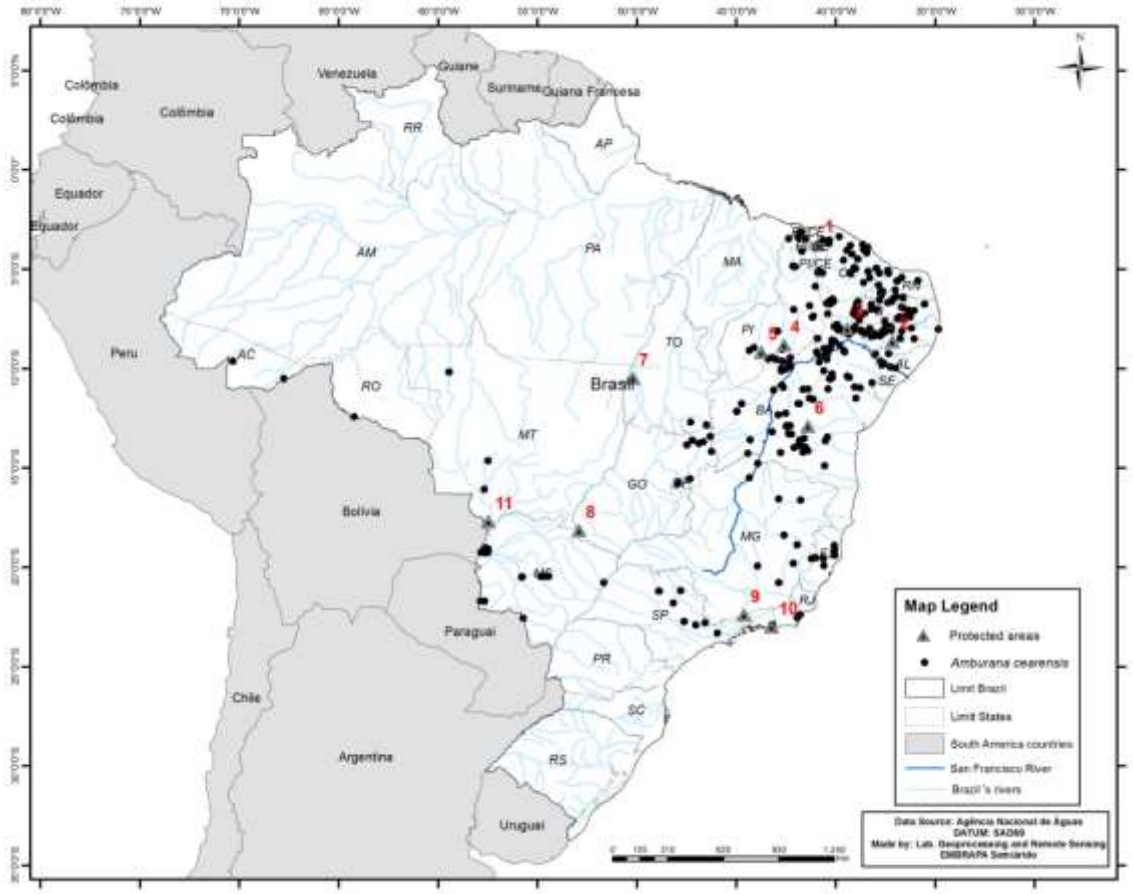


Figure 1. Map of distribution of *A. cearensis* in Brazil and distribution of protected areas: National Park Ubajara (1), National Park Catimbau (2), National Park Flona de Negreiros (3), Serra da Capivara (4), National Park Serra das Confusões (5) and Chapada Diamantina (6) in the Caatinga; the National Parks of Araguaia (7), National Park Emas (8) and National Park Pantanal Matogrossense (11) in the Cerrado; and in the centre-south region, Forest Station of Linhares (10) and the Itatiaia National Park (9) (Made by: Lab Geoprocessing and remote sensing EMBRAPA Semiárido).

In Brazil, the species is found at an altitudinal gradient between 20-800 m a.s.l., in regions where the rainfall and the average annual temperature values can range from 500 to 1700 mm and from 19 to 29 ºC, respectively (CARVALHO, 1994).

In the majority of cases, *A. cearensis* occurs at a terrain constituted by plateaux and its concentration is associated with places of moderately hilly topography with deep richer soils (luvisols) typically found in the Brazilian northeast or in northern Argentina. Occurrences in the Cerrado because of the poor soils are restricted to places with calcareous outcrops where it thrives, although without forming dense or homogeneous stands. It is also associated with rich sandy clayey planosols in Paraguay (LEITE, 2005 and SILVA, 2010).

The climate for the core distribution of the species in the Caatinga ranges from hot, semi-humid tropical (with about 4–5 dry months having less than 50mm mean rainfall) to hot, semi-arid tropical (with dry periods of 6–10 months having less than 50mm mean rainfall) (SALOMÃO; LEITE, 1991). There is a clear pattern of association of the species with lower amounts of rainfall and high temperatures as semi-arid Brazilian northeast (VELLOSO et al., 2001) and southwestern occurrences in Argentina and Paraguay, (LEITE, 2005, Figure 1). However, there are also the occurrence of this species in humid and sub-humid regions (DE SOUZA; FELFILI, 2006 and HAIDAR et al., 2013).

3.1.2 Conservation status

The IUCN Red List of Threatened Species (AMERICAS REGIONAL WORKSHOP, 1996) mentions *A. cearensis* as being endangered due to stands of large trees being destroyed. In Paraguay, the conservation data centre regards the species as threatened (LEITE, 2005). Artificial regeneration by planting of seedlings has been used on a larger scale for this specie (FERREIRA, 2006). Even with this evidence, recently *A. cearensis* has been removed from the Brazilian official list of endangered species that makes more vulnerable to become it extinct.

Trees of this species are found in conservation parks according to figure 1 as the National Parks of Ubajara (1), National Park Catimbau (2), National Park Flona de Negreiros (3), Serra da Capivara (4), National Park Serra das Confusões (5) and Chapada Diamantina (6) representing Caatinga vegetation and great distribution of the species. The National Parks of Araguaia (7), National Park Emas (8) and National Park Pantanal Matogrossense (11) are potentially important conservation areas in the Cerrado region. At the atlantic forest on southeast the taxon is found in the Forest Station Linhares (10) and Itatiaia National Park (9) (LEITE, 2005; Figure 1).

3.1.3 Ethnobotany

In Northeastern Brazil the trade in folk medicinal plants has been practiced since the early 1990s in particular native species (96% of cases), with *A. cearensis* as one of the most commercialized (LIMA; KIILL, 2002). Another aspect that should be highlighted is the use of *A. cearensis* in local commercialization, where bark, leaves, fruits and seeds are sold in local trade.

At the Brazilian Northeast, *A. cearensis* bark is used in folk medicine for preparation of homemade treatments to cure respiratory diseases (BRAGA, 1976). Various substances can be isolated from the bark, such as coumarin, sucrose, two phenol acids (vanillic acid and protocatechuic acid), five flavonoids (afrormosin, isokaempferide, kaempferol, quercetin and 4'-methoxy-fisetin), a phenol glucoside (amburoside A) and a mixture of glucosilated b-sitosterol and stigmasterol (CANUTO; SILVEIRA, 2006). Recent studies show that coumarin, the isokaempferide and the amburoside contain anti-inflammatory, antioxidant and bronchodilator. The isokaempferide and kaempferol contains significant cytotoxic activity against sea urchin eggs and five lineages of the tumour cells (CANUTO; SILVEIRA, 2010).

By virtue of the widespread use of *A. cearensis* for therapeutic purposes, the bronchodilator, analgesic, anti-inflammatory values of the hydroalcoholic extract from the bark of *A. cearensis* was proven to be curative through pre-clinical trial. The extract was shown to be exempt from toxicity at therapeutic doses, ensuring efficacy and safe use in the treatment of various diseases (LEAL et al., 1997 and LEAL et al., 2003). The seeds are oily, providing about 23% of natural oil (MATOS et al., 1992). Seeds are also used as antispasmodic, as emmenagogue and for the treatment of rheumatic diseases, asthma, bronchitis, colds and flu (LORENZI; MATOS, 2002 and MAIA, 2008).

The wood *A. cearensis* is used for high durability furniture, doors and crates (LIMA, 2014) also for barrels of cane sugar cachaça for fast maturation (AQUINO et al., 2005). The seeds are used to produce perfumes and insect repellents (CARVALHO, 1994; CUNHA; FERREIRA, 2003 and MAIA, 2008) and the aqueous extract of *A. cearensis* seed has allelopathic activity inhibiting germination of *Lactuca sativa* L., *Bidens pilosa* L. and *Cenchrus equinatus* L. (BEZERRA et al., 2001 and MANO, 2006).

This species was recommended for projects aiming to restore degraded areas as well as for ornamental and forage purposes (CAMPOS, 2013 and TIGRE, 1968). Sampaio (2006), shows that *A. cearensis* when planted by seedlings have high growth and high survival in the restoration of degraded areas. Venturoli (2011) evaluated, among other species, the survival of *A. cearensis* seedlings in the cerrado biome, suggesting that this specie can be used on a large scale mixing native species.

3.1.4 Flowering, pollination and dispersal

The flowering period of *A. cearensis* in Northeast Brazil occurs between May and July, at the beginning of dry season, and fruiting occurs from August to October, after the loss of their leaves (MAIA, 2008). First flowering and fructification occurs only 10 years after planting (CARVALHO, 1994).

Amburana cearensis is monoecious, with hermaphrodite flowers, also gathered in inflorescences that open during the night. Size of the flowers of *A. cearensis* can be classified as small and medium; the flowers are light-coloured and not very showy. However, in the same inflorescence, the number of buds is very changeable in short times, this variation in the flowers number may enhance the visual appeal for floral visitors at long range, increasing the supply of floral resources available for foraging (KIILL, 2010).

This tree flowers mainly in the dry seasons unlike most plants of Caatinga that have a different phenophase and flower mainly in the wet season. Due to this uncommon flowering season, this species is considered as an important source of pollen and nectar for the local fauna (KIILL, 2010 and SILVA, 2006).

Associated with flowering season is the dispersal of diaspores of each species that can be classified by their morphology and dispersal syndromes into three broad groups: dispersion by wind (anemochory), by animals (zoochory), or without the intervention of external agents (autochory) (MACHADO et al., 1997).

Generally, moths and stingless bees are the pollinators of *A. cearensis*, following the pattern described for the Caatinga where these insect species play a fundamental role in the pollination for most plant species. Flower of *A. cearensis* supply the beehives of native bees in the region during the dry season in which the food sources are scarce (KIILL, 2010).

Fruiting is annual, happening in the dry season and at the beginning of the rainy season. Comparing observations between different years, *A. cearensis* does not have a standard time for development of fruits; for example the fall of the leaves and the fruit production is more accentuated in some years than others (SILVA, 2006).

Dispersal of seeds of *A. cearensis* is anemochorous (seed dispersal by wind) and is favoured by having winged seeds (LORENZI, 2008). As for dispersal distance, the higher number of seed is found on average 4 m from the plant of origin but can be found up to 10 m

(KIILL et al., 2012). These values vary depending on the period of the year, in dry days the dispersion is facilitated by the action of wind where the tree canopy stands out in the landscape (HOWE; SMALLWOOD, 1982).

3.1.5 Harvesting and processing

The fruits are pods, flattened, dehiscent, and release one winged seed per fruit (MATOS et al., 1992). Seeds from green fruits can germinate. However, *A. cearensis* seeds should be harvested when the fruit presents a red colour and before the dehiscence of the seeds, since, in that phase, they are characterized by high germination and vigour due to higher maturity, without any loss in quality and dispersion (SILVA et al., 2014).

Seed harvest is done manually, by picking mature fruits, or by collecting fallen fruits and seeds, by shaking the tree (DANTAS et al., 2012). This is a simple procedure, not requiring skilled labour, although physically strenuous. Depending on the location and characteristics of the tree, the ground should be covered with a canvas to facilitate harvest (SILVA; DANTAS, 2012).

Seeds are processed by drying in shade and removing its wings by manual threshing prior to store (MAIA, 2008 and MATIAS et al., 2014).

3.1.6 Longevity and storage

Having an orthodox behaviour, *A. cearensis* has an initial water content of 5.27% (LÚCIO et al., 2007), and can be stored for longer than 3 months at a sub-zero temperature (LIMA et al., 2008). The plastic container is the most favoured for storage of seeds at ambient or low temperature (cold chamber -10±2 °C) since they have low water content, approximately 5% (DANTAS et al., 2008).

Some fungi as *Penicillium* sp., *Aspergillus* sp., *Rhizopus* sp., *Paecilomyces* sp. are found in seed stored for one year in cold storage (PINHEIRO et al., 2014), requiring, after longer periods of storage, seed treatment with fungicides for germination and seedling production.

3.2 Seedling production

For the production of *A. cearensis* seedlings, germinated seeds can be transferred to forestry trays, polyethylene bags or flowerbeds. Substrates for seed germination may comprise

of soil or soil mixture and sand, soil and commercial substrate, sand and commercial substrate or organic-sand substrate (ROSSI, 2008 and SOUZA et al., 2012). The ground cover can be done by leaves and branches of decomposition of *Mimosa tenuiflora* (Willd.) Poir. and *Croton celtidifolius* Baill. to keep the moisture in the substrate, thus saving irrigation water (PIMENTEL; GUERRA, 2011).

A morphological-anatomical study revealed varying forms of seeds from oblong, elliptical ovoid to slightly compressed. The seed coat has a woody texture, and staining is marbled, rough and opaque. The seed length varies from 12.55 to 17.55 mm and the width varies from 8.35 to 11.50 mm. The hilum is visibly located lateral to the seed base, in a darker and more prominent region. The embryo is axial and cotyledons have an ovoid elliptical shape (BELTRATI et al., 1992 and CUNHA; FERREIRA, 2003; Figure 2A and B).

Seedlings of *A. cearensis* develop an underground hypertrophy, named xylopodium, which contributes to water and supply necessary for the development of the species in the early years of life (LIMA, 1989). This tuber structure of the root is an adaptive strategy, which enables the plant to regrow in case of damage to the above-ground structures (CUNHA; FERREIRA, 2003). The xylopodium presents a fleshy, turnip-shape with red colour. After 9 months, the tuber reaches 3 cm diameter and emits numerous long and thin tuberous roots (CARVALHO, 1994).

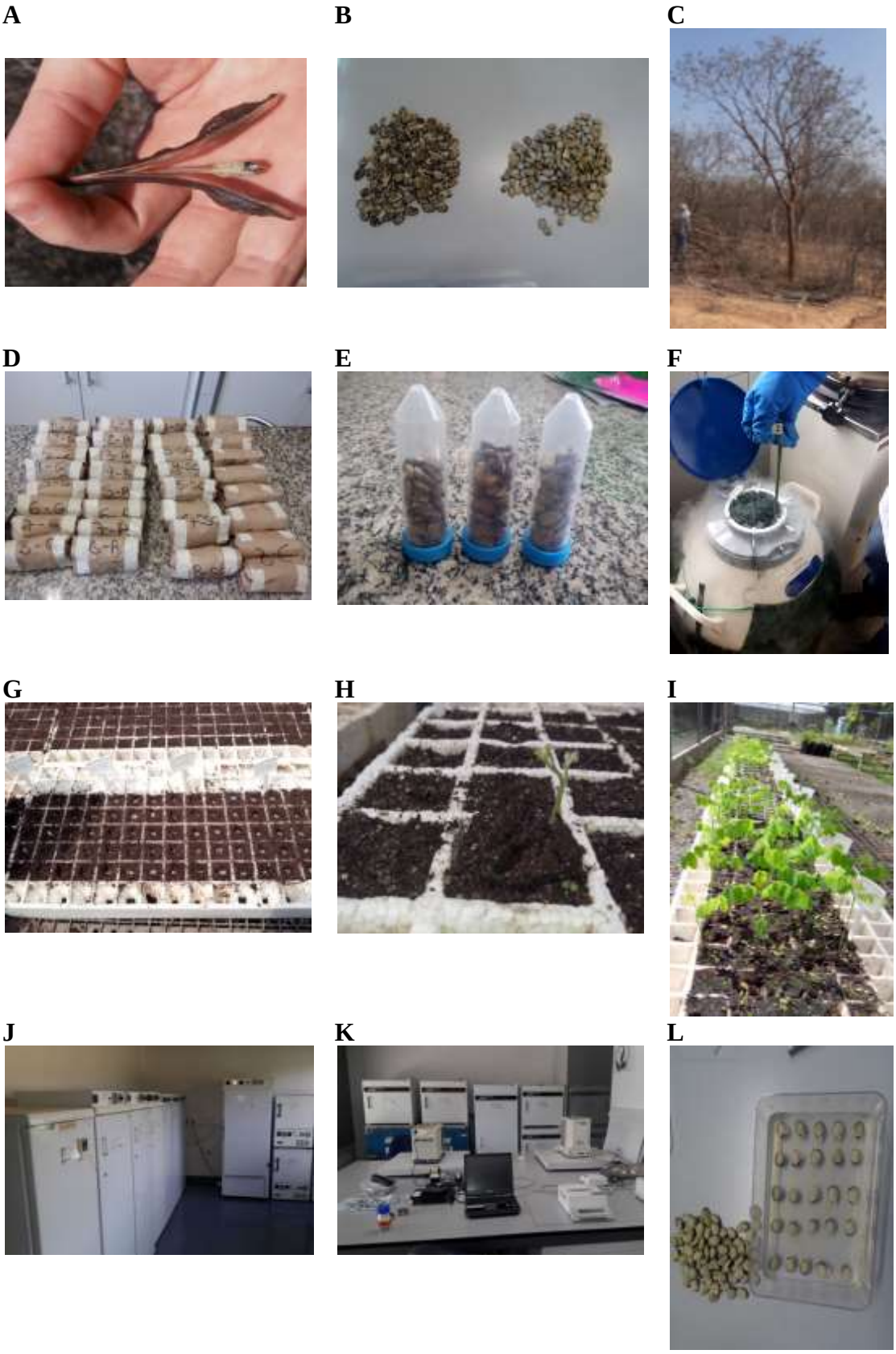


Figure 2. Fruit (A); Seeds (B); Plant habit (C); Seed processing (D); Accessions in laboratory (D); Falcon tubes (E); Container with liquid nitrogen (F); Polystyrene trays (G, H and I); Laboratory of Millennium Seed Bank - Kew (J and K) and Box with sterile agar (L) of *A. cearensis*. Source: author.

3.3 Seed storage

Seed storage is constituted of a set of procedures aimed at preserving their quality in order to provide them with an environment in which the physiological and biochemical changes are maintained at an acceptable level, avoiding unnecessary losses in both the qualitative aspect as the quantitative (BEWLEY et al., 2013). However, the process of deterioration of seeds is inevitable, even when placed in appropriate their preservation environments. Seed quality does not improve during storage, so its initial quality is of fundamental importance for the maintenance of germination and vigour. According Popinigis (1985), the longevity of the seeds is essentially a genetic characteristic. Thus, only the original seed quality and storage of environmental conditions can be manipulated.

In seed conservation studies should consider their physiological behaviour regarding storage. Basically are known three seeds classes in relation to this aspect: the "orthodox" that resist below 10% water content and are able to maintain their viability at temperatures below zero, the intermediate seeds support levels desiccation of between 12 and 15%, however, do not support storage for long periods and temperatures below 15° C, and recalcitrant that do not support the drying under 25 to 50%, with rapid loss of viability (ELLIS et al., 1990; HONG; ELLIS, 2002 and LABBÉ, 2003).

The conservation of seeds can be accomplished in the short, medium and long term, depending on the characteristic of the species. Dehydrated and keeping high germination potential seeds can be stored for long periods (WETZEL, 2012). The metabolic rates of the seeds can be minimized in subzero temperatures, preventing its rapid deterioration (orthodox seeds), which will determine how low the storage temperature can be is the seed water content. According to Bonner (2008) orthodox seeds kept in the water content between 5 and 10% can be safely stored at any temperature.

Changes observed in the seeds behaviour during storage vary depending on the factors that affect conservation, such as temperature, relative humidity, moisture content of the seeds and the type of used packaging (CARNEIRO; AGUIAR, 1991). The degree of importance of these factors in storage and their interactions are a priority to understand the requirements of the species and to maintain its viability.

The temperature affects the respiratory activities of the seeds and growth of microorganisms and reproduction of insects. Conditions dry and cold conditions are more

favourable to orthodox seed storage (MARCOS-FILHO, 2015). The conditions of relative humidity of the storage environment are critical in maintaining the viability of the seeds. If the relative humidity is high in the environment occurs quickly deteriorating seeds. Among the controlled environment conservation systems, the cold chamber are (which retain the seeds at low temperatures and yet high humidity), the dry chamber (which keeps the seeds under relatively low humidity conditions), and camera-cool-dry (which combine low temperatures associated with low relative humidity) (FERREIRA; BORGHETTI, 2004).

3.4 Physiological aspects of germination

Germination is a biological phenomenon that can be considered botanically as the resumption of embryo growth and the consequent disruption of the integument by radical (LABOURIAU, 1983). However, for seed technologists, germination is the emergence and development of key structures of the embryo, demonstrating its ability to produce a normal plant under field conditions (BRASIL, 2013). Germination varies according to seed quality and germination conditions, such as water supply and oxygen and the suitability of temperature, light and substrate. Germination begins with the resumption of metabolic activity, such as activation of enzymes, hydrolysis, assimilation and mobilization of reserves, elongation and cell division, concluding with the root protrusion (CASTRO et al., 2004 and SALOMÃO et al., 2003).

The germination process begins with water uptake by seed tissues, followed by resumption of metabolic activities, particularly the synthesis of new enzymes and increased activities of pre-existing hydrolases, aimed at mobilizing the reserve components of the growth resumption of embryonic axis (BEWLEY; BLACK, 1994; SALES, 2002).

The first condition for the occurrence of germination of a viable and not dormant seed is the availability of water for their rehydration. For this to happen, it is necessary that the seed reach an adequate level of hydration, which allows the reactivation of metabolic processes (POPINIGIS, 1985). Hydration of the seed germination will help to increase respiratory activity to a level capable of sustaining growth of the embryo with the power supply and organic substances (YAP, 1981). Excess moisture generally causes a decrease in germination seen that prevents the penetration of oxygen and reduces all the resulting metabolic process (CARVALHO; NAKAGAWA, 2012). Adequate moisture is variable between species (MARCOS-FILHO, 2015).

The seeds water uptake process it is followed by a three-phase model (BEWLEY; BLACK, 1994). Phase 1 is a physical process where there is a rapid water uptake by seeds, regardless of the material is alive or not. Subsequently, when a reduction in imbibition speed and respiratory intensity occurs, starts the Phase 2. At the phase 2, the metabolic processes essential for embryonic growth, are intensified and germination is complete with radicle protrusion, initiating phase 3. Phase 2 and phase 3 are steps achieved only by living seeds and non dormant (SOUZA, 2009). The duration of each of these phases of imbibition depends on certain inherent properties of the seed (e.g., hydratable substrate content, seed coat permeability, seed size) and on the prevailing conditions during hydration (e.g., temperature, initial moisture content, water and oxygen availability) (BEWLEY et al., 2013).

Temperature is a factor that influences not only in the germination of the seeds, but also the water absorption speed and biochemical reactions that determine the whole process. Germination involves a sequence of biochemical reactions by which reserve substances stored in seeds are broken down, mobilized and resynthesized. Similarly to a chemical reaction, germination is much faster and more efficient process when are in higher temperature, to some extent (CARVALHO; NAKAGAWA, 2012).

The optimum temperature for the majority of plant species is between 20 to 30 °C and a maximum between 35 °C and 40 °C (MARCOS-FILHO, 2015). The range 20 ºC to 30 °C was also considered by Borges and Rena (1993) as the most suitable for the germination of a great number of tropical and subtropical tree species.

Seed quality includes a number of characteristics and attributes that determine its value for sowing, among the most relevant characteristics are considered genetic, physical, physiological and sanitary that influence in the seed ability to give powerful and representative plants species (MAIA et al., 2007). And the knowledge of how environmental factors influence the germination of seeds is extremely important, and can be controlled and manipulated in order to increase the seed vigour, resulting in production of more vigorous seedlings and better development (NASSIF et al., 1997).

Seed vigour is a reflection of the set of characteristics that determine their physiological potential, that is, the ability to present an adequate performance when exposed to different environmental conditions. The loss of seed vigour is related to the early events of decay sequence, which provides physiological, biochemical, physical and cytological changes, culminating with the seed of death (MARCOS-FILHO, 2015).

During germination soluble reserves of high molecular weight present in the seeds, such as lipids, proteins and sugars are degraded and converted to soluble forms which are quickly transported to tissue growth and used in synthesis or energy production reactions. The metabolic changes that occur in these stages are the result of the activity of various enzymes hydrolysis and transfer (BEWLEY; BLACK, 1994 and BUCKERIDGE et al., 2004) and may express the physiological seed quality.

The main carbohydrates that act as reserves of seeds are sucrose, oligosaccharide (raffinose), starch and cell wall polysaccharides. While sucrose is nearly universal, oligosaccharide (raffinose) occurs at a large number of dicotyledonous seeds. Starch is a natural, renewable, biodegradable polysaccharide produced by many plants as a storage polymer and cell wall polysaccharides occur in some taxonomic groups which generally act as reserve, but preserving important secondary functions such as absorption and control of water distribution in different tissues of seeds. While the main function of oligosaccharides are attributed to the ownership of orthodox seeds to stabilize their membranes and, therefore, may remain dry for a long period, after which usually germinate when exposed to liquid environments (BUCKERIDGE et al., 2004).

A. cearensis is classified as orthodox species since seeds are tolerant to drying and can be stored with moisture content around 8% without rapid loss of viability (FIGLIOLIA, 1988 and GUEDES et al., 2010b). The initial imbibition of seeds is slow (LUZ, et al., 2004), however, this species does not present dormancy and germinates readily under favourable environmental conditions. Radicle emergence start after 5 days, seedling emergence in substrate starts to after 12 days and seedling growth is usually observed after 15 days (BRASIL, 2013; LÚCIO et al., 2006 and OLIVEIRA et al., 2014).

Germination of *A. cearensis* begins with the rupture of the seed coat in the base near the hilum. The primary root has a simple bristle, then gets brown yellow placement, starting the formation of secondary roots. The hypocotyl is short and the cotyledons break the skin on the opposite and unilateral sense. The epicotyl is visible from the 8th day of sowing. The apical bud presents well developed since the beginning of germination and can be seen when it promotes the opening of the cotyledons (CUNHA; FERREIRA, 2003). According to Miquel (1987) classification, the species has germination of semi-hypogeal phanerocotylar type.

According to the literature, in laboratory, the optimal germination temperature on paper substrate moistened with water is between 30-35 ºC with a water volume from 2.5 to 3.5 times

the weight of the paper and a 12/12 h photoperiod (ALMEIDA et al., 2014; BRASIL, 2013; GUEDES et al., 2010a and OLIVEIRA et al., 2014).

3.5 Mathematical models in germination

In Brazil, the use of thermal mathematical models has been not very widespread to develop temperature (*T*) response germination patterns. Temperature has a fundamental influence on germination, dormancy regulation, rate or speed of germination in quiescent seeds, removing of primary and/or secondary dormancy and inducing secondary dormancy (BEWLEY et al., 2013).

Since 1800s three cardinal temperatures have been recognized to describe the range of *T* over which seeds of a particular species can germinate: minimum or base temperature (*Tb*) that is the lowest *T* at which germination can occur; optimum temperature (*To*) which is the *T* at which germination is most rapid and maximum; and the maximum or ceiling temperature (*Tc*) meaning the highest *T* at which seeds can germinate (BEWLEY et al., 2013; GARCIA-HUIDOBRO et al., 1982; GUMMERSON, 1986).

The cardinal temperatures for germination are generally related to the environmental range of adaptation of a given species and serve to match germination timing to favourable conditions for subsequent seedling growth and development (ALVARADO; BRADFORD, 2002). The temperature range between *Tb* and *Tc* is sensitive to the dormancy status of the seeds, often being narrow in dormant seeds and widening as dormancy is lost (BEWLEY et al., 2013). In particular, low *Tc* values are often associated with seed dormancy, as in relative dormancy or thermo-inhibition exhibited by seeds whose germination is prevented at warm temperatures (BRADFORD; SOMASCO, 1994).

Thermal time has been used to analyse the effects of temperature the germination of seeds (TRUDGILL et al., 2005). A common approach for expressing the relationship between temperature and plant development is to calculate the thermal time (*Tt*). In its simplest form *Tt* is calculated as the mean temperature minus the base (*Tb*) or threshold temperature below which no development takes place and is given by the reciprocal of the slope of the regression (MOOT et al., 2000; TRUDGILL, et al., 2000).

For the suboptimal temperature range (between *Tb* and *To*) this relationship can be described mathematically as:

$$\theta_T(g) = (T - Tb)t_g$$

Where θT(g) is the thermal time to germination of fraction or percentage g, *T* is the germination temperature, *Tb* is the base temperature.

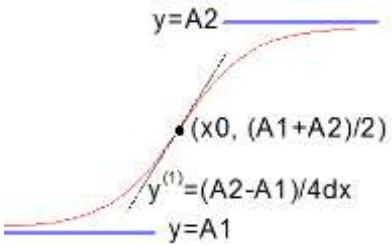
Time to 50% germination (t_{50}) is also calculated according to the following equation:

$$t_{50} = t_j + \left[\frac{(N+1)/2 - ni}{nj - ni}\right].(t_j - t_i)$$

Where *N* is the final number of seeds germinating and *ni*, *nj*, total number of seeds germinated by adjacent counts at time t_i, t_j, where $n_i < (N + 1)/2 < n_j$.

Using time-course cumulative germination curves adjusted by Boltzmann function, parameters such as t_{50} also can be done:

$$y = \frac{A1 - A2}{1 + exp^{(x-x0)/dx}} + A2$$



Were *A1* is initial value, *A2* final value, *x0* means center or time to reach 50% (t_{50}) and *dx* time constant.

Germination rate is the reciprocal of time to germination for specific germination percentages (usually 50%) and is very sensitive to temperature, generally increasing with temperature to an optimum and then decreasing sharply at temperatures above the optimum.

Thus, created the GR concept.

$$GR = {}^{1}/_{t_{50}}$$

Between the sub- and supra-optimal and the optimum condition, germination rates increase linearly with an increase in water potential and temperature (GUMMERSON, 1986). Thus, time required for germination is a function of the length of time seeds have received water potentials and temperatures above the base (but not above the optimum) (ROWSE; FINCH-SAVAGE, 2003).

Although total germination percentages tend to show a broad maximal range, germination rates more narrowly identify the optimum temperature for germination. Germination rates of more dormant seed populations may also be slower compared to less dormant seeds at the same temperature (BEWLEY et al., 2013).

Uniformity of germination is indicated by the time between two germination percentiles, such as the time between 10 and 90% (RAHIMI, 2013), 20 and 80% (BEWLEY et al., 2013) or between 25 and 75% germination (KHAN et al., 2012); smaller values indicate greater uniformity. Statistically, uniformity of germination illustrates germination spreading over the time.

3.6 Environmental stresses

Tropical plants of semi-arid regions are subject to adverse environmental conditions, among them to water stress, soil salinity and extreme temperatures (YANCEY et al., 1982). For germination to occur satisfactorily, the seeds must have essential conditions such as water, oxygen and temperature. The degree requirement of these factors varies among species and is determined by the genotype and the prevailing environmental conditions during seed formation (MAYER; POLJAKOFF-MAYBER, 1989).

The ability of the plants to maintain the fluid status of the cells (osmotic adjustment) and cell integrity in semi-arid regions can be an adaptive advantage (JONES; CORLETT, 1992). The availability of water is able to influence the germination process and post-germinating seedling development. This condition is seen as a limiting factor to the initiation of seed germination and seedling establishment in the field. Because it directly affects the water relations in seeds and the subsequent development of seedlings, resulting directly or indirectly in all other stages of metabolism, including reactivation of the cell cycle and growth (CASTRO et al., 2000 and ROCHA, 1996).

The high salt content in the soil, especially sodium chloride (NaCl), can inhibit the germination, primarily due to osmotic effect (FANTI; PEREZ, 1996). Also, the increase in salt concentration produces an increase in the percentage of abnormal seedlings, because the toxic effects of salts on seeds (CAMPOS; ASSUNÇÃO, 1990). The growth and survival of plants to high salt conditions depend adaptation to low water potential and high concentrations of sodium. Three aspects are relevant to the tolerance of plants to salt: (1) ion homeostasis, (2) detoxification and (3) control of growth (ZHU, 2001).

Temperature influences the metabolism of seeds, altering biochemical or physiological processes and is responsible not only for the germination rate but also by the end of germination percentage (BEWLEY; BLACK, 2012). Each species has a range of temperatures at which germination will occur, although the range of 20 ºC to 30 ºC shows is suitable for germination of many subtropical and tropical species (BORGES; RENA, 1993). The optimum temperature provides the maximum percentage of germination in the shortest time (BEWLEY; BLACK, 1994).

4.0 CHAPTER 1

INFLUENCE OF THE STORAGE CONDITION ON SEED QUALITY OF *Amburana cearensis* (Allemão) A.C.Sm. (Fabaceae)

ABSTRACT: The aim of this work was to evaluate effects of storage conditions on germination of *A. cearensis* seeds. The experimental design was completely randomized in split-plots along time with four replicates. Storage conditions as airtight container in refrigerator; airtight container in laboratory, paper bags in laboratory and liquid nitrogen were assessed during 27 months. In laboratory we evaluated germination, germination rate, uniformity germination, total soluble and reducing sugars in radicle. In the greenhouse were evaluated seedling emergence, emergence rate and 30 days seedlings height. Seed stored in refrigerator maintained high initial germination and decreased from 21th month. Seeds storage in paper bags in laboratory presented low emergence and smaller seedlings. Total soluble sugars and reducing sugars presented decreased until 21th month, followed by increased until the last accessed month. It is not advisable to store *A. cearensis* seeds in laboratory environment without an airtight container. *A. cearensis* seeds kept in refrigerated environment maintain the viability for at least two years.

Keywords: Caatinga, conservation, emergence, Leguminosae, umburana-de-cheiro

RESUMO: O objetivo deste trabalho foi avaliar os efeitos das condições de armazenamento sobre a germinação de sementes de *A. cearensis*. O delineamento experimental foi inteiramente casualizado em parcelas subdivididas ao longo do tempo com quatro repetições. As condições de armazenamento como recipiente hermético no refrigerador; recipiente hermético em laboratório, sacos de papel em laboratório e nitrogênio líquido foram avaliadas durante 27 meses. No laboratório foram avaliados germinação, taxa de germinação, uniformidade de germinação, açúcares solúveis totais e redutores da radícula. Em casa de vegetação avaliou-se emergência das plântulas, taxa de emergência e altura de mudas no decorrer dos 30 dias. As sementes armazenadas no refrigerador mantiveram alta germinação inicial e diminuíram a partir do 21º mês. O armazenamento de sementes em sacos de papel em laboratório apresentou baixa emergência e menores mudas. Os açúcares solúveis totais e açúcares redutores apresentaram diminuição até o 21º mês, seguido de aumento até o último mês analisado. Não é aconselhável armazenar sementes de *A. cearensis* em ambiente de laboratório sem um recipiente hermético. As sementes de *A. cearensis* mantidas em ambiente refrigerado mantêm a viabilidade durante pelo menos dois anos.

Palavras-chave: Caatinga, conservação, emergência, Leguminosae, umburana-de-cheiro

Introduction

Caatinga biome (Brazilian semiarid vegetation) has a significant biological diversity compared to other semiarid regions of the world. This biodiversity is extremely important for local communities to whom this biome provides timber, food, medicine and forage (LOIOLA et al., 2012; SANTOS et al., 2011 and SANTOS et al., 2010). Uncontrolled exploitation of natural resources of Caatinga caused severe degradation of vegetation, mainly due to deforestation for agricultural activities, without allowing the species regeneration or reforestation (FARIAS et al., 2013).

Climate of Caatinga presents temperature with little variation and rainfall usually totals less th an 750 mm/year, deeply affecting the plant species living in the region with average temperatures approximately 26 °C (COSTA et al., 2007). Vegetation is conditioned to water deficit mainly related to irregularity of rains associated with high temperatures, high light intensity, which cause a high evaporative demand and consequent desiccation of the soil (TROVÃO et al., 2007). This climatic instability, together with human occupation, threatens the native biodiversity of Caatinga (LEAL et al., 2005 and LIMA-ARAÚJO et al., 2007). Thereby, great part of Caatinga has suffered from drought since 2011 (LEIVAS et al., 2014). And this can cause damage for seedlings to settle with few rainy periods.

Amburana cearensis (Arr. Cam.) A.C. Smith (Fabaceae) is a tree native from South-America and typical of Caatinga biome and is often explored by local populations as medicinal potential leading this species to extinction (PIMENTEL; GUERRA, 2010). *A. cearensis* is known for its medicinal properties: bark and seeds are used to produce popular medications to treat pulmonary diseases, cough, asthma, bronchitis and whooping cough (MAIA, 2008). This is one of reasons why *A. cearensis* is currently listed in the global IUCN list as an endangered species and was listed until 2015 in the Brazilian national list of endangered species (AMERICAS REGIONAL WORKSHOP, 1998).

Seed deterioration process is inevitable, even when placed in appropriate preservation environments (ARJMAND et al., 2014). Factors such as temperature and humidity can influence the process of seed deterioration during storage (MONCALEANO-ESCANDON et al., 2013). Therefore, it is utterly important to provide to all species efficient methods and conditions to store seeds to maintain their viability. Thus, in endangered species, there is an urgent need to determine seed conservation strategies involving the maintenance of a high level of seed germination, seedling establishment and the preservation of the physiological potential, during seed storage. Some studies have reported alternative storage conditions for Caatinga

species seeds such as *Caesalpinia pyramidalis* (OLIVEIRA et al., 2012), *Myracrodruon urundeuva* (GUEDES et al., 2012b), *Caesalpinia leiostachya* (BIRUEL et al., 2007). That shows oscillations in seed vigour by the different ways of packing seeds for storage.

To maintain the quality of stored seeds, factors such as seed moisture and storage temperature are important to maintenance of seeds quality. Since during the storage period, seeds quality cannot be improved, but can be maintained for a long period (ZUCHI et al., 2013). In addition, in order to better understanding the seed behaviour in storage, it is essential to verify factors such as resistances of these species at low temperatures.

Thereby, in order to evaluate the storage performance for a medium period, this study aimed to evaluate the germination of *A. cearensis* seeds in different storage conditions.

Materials and Methods

Seeds of *Amburana cearensis* used in this experiment were harvested in Caatinga biome in Lagoa Grande, state of Pernambuco (S 8º34’04,00’’; O 040º10’18,00’’; Figure 3) from dehiscent fruits in August 2013. Fresh seeds were readily evaluated for seed qualities were compared with the stored seeds.

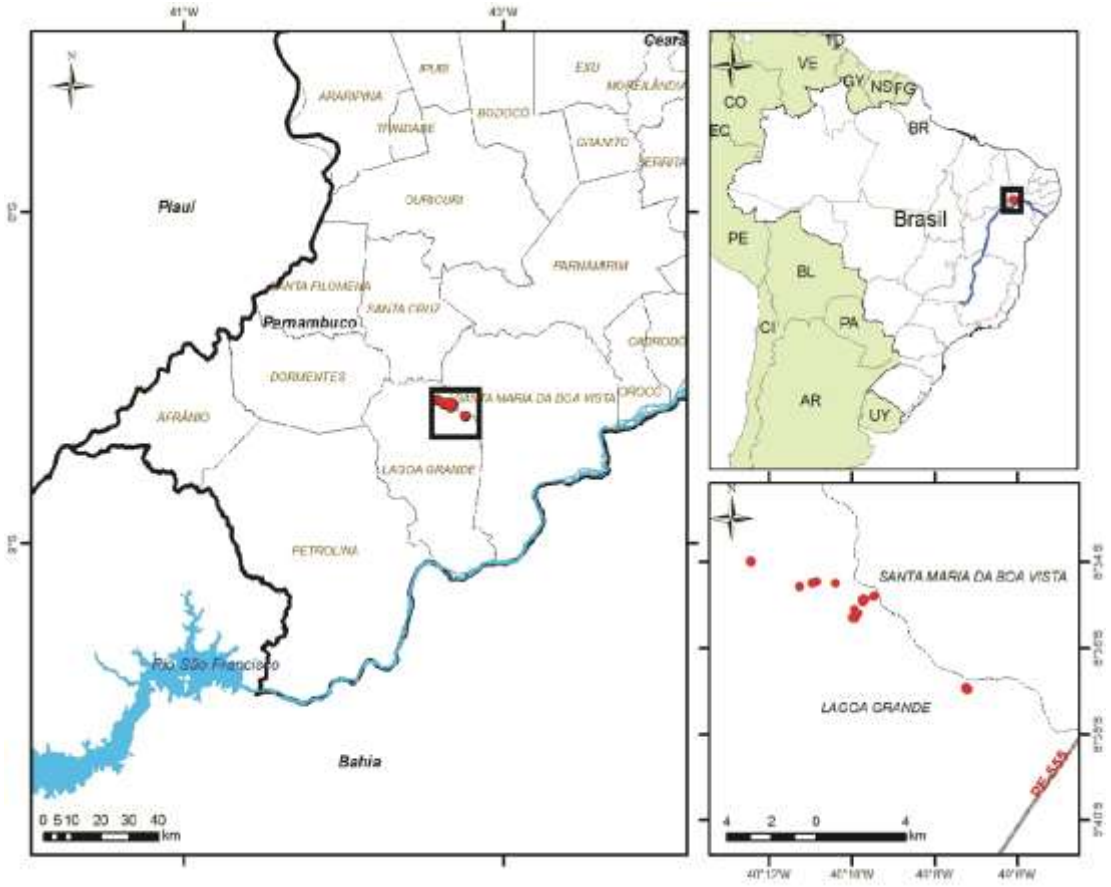


Figure 3. Map of the collecting area (square) in Lagoa Grande / Pernambuco state in Brazil (Made by: Lab Geoprocessing and remote sensing EMBRAPA Semiárido)

The experimental design was completely randomized in split-plots along time, with four replicates. Four different storage conditions were considered as plots and storage time was considered as subplots.

Seed storage: seeds were stored in four different conditions such as: craft paper bags enclosed in airtight containers in refrigerator (4±3 °C, 60±4% RH); craft paper bags enclosed in airtight container in a laboratory environment (25±4 °C, 19±3% RH); craft paper bags in laboratory environment (25±4 °C, 56±6% RH; Figure 2D) and polypropylene tubes in liquid nitrogen (-196 °C; Figure 2E and F). Seeds remained in these conditions for 27 months. Seed samples were removed from each storage condition in order to evaluate seeds quality. Temperature and relative humidity were monitored with a data logger - Hobo data logger - model U10-003.

Before storage all seeds were put in container with silica gel for 60 min in order to standardize the water content in approximately 9%. Seeds in cryopreservation were placed in Falcon tubes followed placed in a container with liquid nitrogen. Seeds removed from liquid

nitrogen were immediately placed in refrigerator (5±3 °C, 60±4% UR) for 60 min, allowing gradual thawing and relatively rewarming of samples (PRITCHARD; NADARAJAN, 2008).

In order to evaluate fresh and stored seeds quality, four replicates of 25 seeds were used in germination test, seedling emergence test and to quantify sugar metabolism in germinating seeds during 27 months.

Water content: It was obtained by oven method at 105±3 °C for 24 hours, using two samples of 10 seeds and the results expressed as a percentage based on seed fresh weight (BRASIL, 2013).

Germination test: It was carried out on germination paper soaked with distilled water at a proportion of 2.5 times the dry paper weight. Seeds were germinated in BOD chamber at 30 °C and 12 hours photoperiod (BRASIL, 2013). Seed germination scoring was performed daily until the seedling establishment, which occurred approximately 15 days. The seeds were considered as germinated at 1mm radicle emergence.

Final germination (FG, %); germination uniformity (time elapsed between 20% and 80% germination, GU, $days^{-2}$) and germination rate (reciprocal of time to reach 50% of final germination, GR, $days^{-1}$) were estimated (TOOROP et al., 2012).

Seedling emergence test: It was performed sowing, fresh and stored seeds in polystyrene trays containing commercial substrate Plantmax® and arranged in greenhouse with controlled environment (40% luminosity with black shading screens and manual irrigation according to plant requirements; Figure 2G, H and I). The emergence was daily evaluated during 30 days (BRASIL, 2013) and final emergence (FE, %); emergence rate (reciprocal of time to reach 50% of final emergence, ER, $days^{-1}$) and average 30 days seedling height (SH) were calculated.

Total soluble sugars and reducing sugars quantification: extractions were performed by grounding four replications of 0.5 g root samples (c. 10 seedlings) in a sterile mortar with 10 ml of distilled water. The mixture was centrifuged at 3.000 *x*g for 20 minutes without refrigeration. The supernatant was collected to microtubes and kept in a freezer at -20 °C until reducing sugars (MILLER, 1959) and total soluble sugars (MORRIS, 1948 and YEMM; WILLIS, 1954) assays.

Statistical analysis: data were tested for normality and homogeneity of variance before comparing means through the tests of Shapiro-Wilk and Levene's test both at 0.05 probability

level. Non-normal percentage data were arcsine-transformed and re-tested. Continuing non-normal data were analyzed by non-parametric test of Kruskal-Wallis at 0.05 probability level. For normal data, Tukey test were used at 0.05 probability level and fresh seeds were compared with stored seeds by Dunnett test at 0.05 probability level.

Results

A. cearensis seeds presented initial 9.2% water content, which did not change during storage, regardless the condition.

Germination (FG), germination rate (GR), emergence (FE), emergence rate (ER) and total soluble sugars (TSS) data were not normally distributed and/or not homogeneous and therefore the media test used was Kruskal-Wallis.

Storage conditions influenced germination behaviour of *A. cearensis* seeds. Seeds from laboratory environment packed only in paper bags showed decreased for FG in the 27th month differing statistically from fresh seeds and 6 month of storage. Seeds kept in refrigerator and laboratory both in airtight containers did not show germination differences to fresh seeds between them and over time by Kruskal-Wallis test (Table 1).

Table 1. Final germination (%) of *A. cearensis* seeds in different storage conditions and times of storages.

Time Storage (Months)	**Storage conditions**			
	Airtight container in Refrigerator	**Airtight container in Laboratory**	**Laboratory without container**	**Liquid nitrogen (N_2) container**
0 98.0				
6	90.0 Aa	87.5 Aa	98.0 Aa	87.0 Aa
9	93.0 Aa	93.0 Aa	93.0 Aab	83.0 Aa
12	94.0 Aa	91.0 Aa	94.0 Aab	87.0 Aa
21	92.7 Aa	90.0 Aa	82.0 Aab	85.0 Aa
24	94.0 Aa	95.0 Aa	83.0 ABab	•72.0 Ba
27	90.7 Aa	90.0 Aa	•76.0 Ab	90.0 Aa

CV%[a] = 7.70; **W**= 0.98ns; **F**= 2.29**

Means followed by the same letter capital letters in the line and lowercase on the column do not differ by Kruskal-Wallis ranking values at 5% probability. Means followed by • differ from the initial time (fresh seeds) by Kruskal-Wallis test at 5% probability. **W**; **F**: statistics of Shapiro-Wilk and Levene's test respectively indicate residue with normal distribution and variance. Ns and ** = not significant and significant at 1%, respectively.

Seeds stored in liquid nitrogen also differed from fresh seeds in the 24th month for FG and as of 12th month for GU (Tables 1 and 3) according to Kruskal-Wallis and Dunnett test respectively.

Amburana cearensis seeds in laboratory without container showed decreased for GR (high speed germination) differing statistically from seeds kept in airtight container in laboratory in the 27th month of storage. Except for the 12th month in seeds stored in laboratory without container (which can be attributed to an outlier), all others did not show GR differences to fresh seeds by Kruskal-Wallis test (Table 2).

Table 2. Germination rate (dias^{-1}) of *A. cearensis* seeds in different storage conditions and times of storage.

Time Storage (Months)	Storage conditions			
	Airtight container in Refrigerator	**Airtight container in Laboratory**	**Laboratory without container**	**Liquid nitrogen (N_2) container**
0	0.188			
6	0.154 ABa	0.150 ABa	0.185 Aab	0.143 Ba
9	0.155 Aa	0.181 Aa	0.195 Aab	0.173 Aa
12	0.174 ABa	0.198 ABa	•0.236 Aa	0.162 Ba
21	0.152 Aa	0.153 Aa	0.150 Aab	0.164 Aa
24	0.170 Aa	0.171 Aa	0.147 Ab	0.144 Aa
27	0.170 ABa	0.177 Aa	0.130 Bb	0.142 ABa
CV% = 10.28; **W**= 0.97*; **F**= 1.87*				

Means followed by the same letter capital letters in the line and lowercase on the column do not differ by Kruskal-Wallis ranking values at 5% probability. Means followed by • differ from the initial time (fresh seeds) by Kruskal-Wallis test at 5% probability. **W**; **F**: statistics of Shapiro-Wilk and Levene's test respectively indicate residue with normal distribution and variance. * = significant at 5%.

Table 3. Germination uniformity (dia^{-2}) of *A. cearensis* seeds in different storage conditions and times of storage.

Time Storage (Months)	Storage conditions			
	Airtight container in Refrigerator	**Airtight container in Laboratory**	**Laboratory without container**	**Liquid nitrogen (N_2) container**
0	2.53			
6	3.61 Aa	3.69 Aa	2.62 Aa	3.70 Aa
9	4.36 Aa	3.45 Aa	3.53 Aa	3.96 Aa
12	3.85 Aa	3.24 Aa	1.95 Aa	•5.18 Aa
21	4.34 Aa	3.57 Aa	2.82 Aa	•5.10 Aa
24	3.60 Aa	3.04 Aa	3.19 Aa	•4.87 Aa
27	4.07 Aa	2.46 Aa	3.70 Aa	•4.96 Aa
CV% = 23.80; **W**= 0.99ns; **F**= 1.39ns				

Means followed by the same letter capital letters in the line and lowercase on the column do not differ by Tukey test at 5% probability. Means followed by • differ from the initial time (fresh seeds) by Dunnett test at 5% probability. **W**; **F**: statistics of Shapiro-Wilk and Levene's test respectively indicate residue with normal distribution and variance. ns = not significant.

FE percentage in greenhouse conditions of *A. cearensis* stored seeds shows that refrigerator stored seeds maintained their vigour in comparison to fresh seeds and over storage time. Seeds stored in laboratory environment packed in paper bags and in liquid nitrogen

container showed lower FE percentage than fresh seeds as of 21th month of storage. Following similar behaviour, *A. cearensis* seeds kept in airtight container in laboratory environment showed reduction in the values as of 21th month of storage with differences to fresh seeds in the 24th and 27th months by the no parametric Kruskal-Wallis test (Table 4).

Table 4. Final emergence (%) of *A. cearensis* seeds in different storage conditions and times of storage.

Time Storage (Months)	Storage conditions			
	Airtight container in Refrigerator	**Airtight container in Laboratory**	**Laboratory without container**	**Liquid nitrogen (N_2) container**
0	81.25			
6	80.0 Aa	62.5 ABa	43.7 Ba	56.7 ABa
9	77.5 Aa	61.2 Aa	48.8 Aa	56.7 Aa
12	77.5 Aa	72.5 Aa	48.7 Aa	63.7 Aa
21	64.4 Aa	•31.4 Aa	•30.5 Aa	•35.0 Aa
24	65.7 Aa	40.0 ABa	•21.5 Ba	•38.7 ABa
27	63.0 Aa	•35.0 ABa	•20.0 Ba	•33.7 ABa
CV% = 25.16; **W**= 0.97*; **F**= 2.04*				

Means followed by the same letter capital letters in the line and lowercase on the column do not differ by Kruskal-Wallis ranking values at 5% probability. Means followed by • differ from the initial time (fresh seeds) by Kruskal-Wallis test at 5% probability. **W**; **F**: statistics of Shapiro-Wilk and Levene's test respectively indicate residue with normal distribution and variance. * = significant at 5%.

Regarding ER and seedlings height (SH) of stored seeds in laboratory without container the latter two storage evaluations statistically differed to fresh seeds and to the former two storage evaluations (Tables 5 and 6). We also noticed this trend in seeds kept in container in laboratory for SH (Table 6).

In all storage conditions seedlings' roots of *A. cearensis* showed a slight decrease in TSS contents until 21th month with lower values followed by an increase up to the last evaluation month. Among all storage conditions, have not been observed differences of levels of TSS when compared to fresh seeds by Kruskal-Wallis test (Table 7).

Seeds in N_2 liquid also differed to the former two storage evaluations for ER and SH, but only the 12th month for ER and the latest storage evaluation of SH differed to fresh seeds (Table 5 and 6).

Table 5. Emergence rate (dias^{-1}) of *A. cearensis* seeds in different storage conditions and times of storage.

Time Storage (Months)	Storage conditions			
	Airtight container in Refrigerator	**Airtight container in Laboratory**	**Laboratory without container**	**Liquid nitrogen (N_2) container**
0	0.0668			
6	0.0660 ABa	0.0698 ABab	0.0720 Aa	0.0623 Ba
9	0.0679 Aa	0.0728 Aa	0.0741 Aa	0.0681 Aa
12	0.0597 Aa	0.0594 Ab	0.0585 Aab	•0.0552 Aab
21	0.0628 Aa	0.0594 Aab	0.0585 Aab	0.0650 Aab
24	0.0560 Aa	0.0561 Aab	•0.0485 Bb	0.0581 Ab
27	0.0571 ABa	0.0594 Aab	•0.0487 Bb	0.0596 ABb

CV% = 6.50; **W**= 0.97*; **F**= 1.90*

Means followed by the same letter capital letters in the line and lowercase on the column do not differ by Kruskal-Wallis ranking values at 5% probability. Means followed by • differ from the initial time (fresh seeds) by Kruskal-Wallis test at 5% probability. **W**; **F**: statistics of Shapiro-Wilk and Levene's test respectively indicate residue with normal distribution and variance. * = significant at 5%.

Table 6. Seedling height (cm^{-1}) of *A. cearensis* seeds under different storage conditions and times of storage.

Time Storage (Months)	Storage conditions			
	Airtight container in Refrigerator	**Airtight container in Laboratory**	**Laboratory without container**	**Liquid nitrogen (N_2) container**
0	12.4			
6	10.8 Bb	12.9 Aab	12.9 Aab	12.5 Aab
9	13.8 Aa	14.1 Aa	14.6 Aa	14.4 Aa
12	12.3 Aab	11.1 Abc	12.0 Ab	11.5 Abc
21	12.1 Aab	12.9 Aab	11.2 Ab	12.8 Aab
24	10.7 Ab	•9.4 ABc	•7.8 Bc	10.6 Abc
27	11.1 Ab	•9.8 ABc	•8.1 Bc	•9.9 ABc

CV%[a] = 9.60; **W**= 0.98ns; **F**= 1.49ns

Means followed by the same letter capital letters in the line and lowercase on the column do not differ by Tukey test at 5% probability. Means followed by • differ from the initial time (fresh seeds) by Dunnett test at 5% probability. **W**; **F**: statistics of Shapiro-Wilk and Levene's test respectively indicate residue with normal distribution and variance. ns = not significant.

Reducing sugars (RS) contents in liquid nitrogen stored seeds reached higher values as of 21 months of storage compared to roots of fresh seeds by Dunnett test and to seed' roots stored in laboratory without container by Tukey test. RS content of seed' roots stored in laboratory without container from 12th month had values significantly down comparing to the former two storage evaluations (Table 8).

Table 7. Total soluble sugars (TSS, µmol.mg^{-1}.fw) of *A. cearensis* seedlings' roots in different storage conditions and times of storage.

Time Storage (Months)	Storage conditions			
	Airtight container in Refrigerator	Airtight container in Laboratory	Laboratory without container	Liquid nitrogen (N_2) container
0	392.8			
6	503.6 Aab	383.6 Aab	417.8 Aa	444.0 Aa
9	457.6 Aa	348.0 Bb	401.0 ABa	415.6 ABab
12	392.4 Aab	410.0 Aab	369.2 Aa	366.2 Aab
21	360.0 Aab	335.8 ABb	311.6 ABa	284.8 Bb
24	380.2 Ab	380.4 Aab	323.4 Aa	343.4 Aab
27	448.6 Aab	487.2 Aa	411.0 Aa	380.0 Aab

CV% = 12.75; **W**= 0.98ns; **F**= 3.43**

Means followed by the same letter capital letters in the line and lowercase on the column do not differ by Kruskal-Wallis ranking values at 5% probability. Means followed by • differ from the initial time (fresh seeds) by Kruskal-Wallis test at 5% probability. **W**; **F**: statistics of Shapiro-Wilk and Levene's test respectively indicate residue with normal distribution and variance. ns and ** = not significant and significant at 1%, respectively.

Table 8. Reducing sugar (RS, µmol.mg^{-1}.fw) of *A. cearensis* seedlings' roots in different storage conditions and times of storage.

Time Storage (Months)	Storage conditions			
	Airtight container in Refrigerator	Airtight container in Laboratory	Laboratory without container	Liquid nitrogen (N_2) container
0	177,9			
6	•273.5 Aa	185.4 Bab	229.6 ABa	228.2 ABbc
9	•246.3 Aab	202.4 Aa	•240.7 Aa	207.2 Acd
12	158.2 Ad	179.7 Aab	153.7 Ab	156.9 Ad
21	177.8 Bcd	148.9 Bb	160.7 Bb	•276.2 Aab
24	166.6 Bcd	207.5 Ba	172.5 Bb	•295.6 Aa
27	212.8 Abc	215.5 Aa	159.4 Bb	•260.56 Aabc

CV%[a] = 13.48; **W**= 0.99ns; **F**= 1.39ns

Means followed by the same letter capital letters in the line and lowercase on the column do not differ by Tukey test at 5% probability. Means followed by • differ from the initial time (fresh seeds) by Dunnett test at 5% probability. **W**; **F**: statistics of Shapiro-Wilk and Levene's test respectively indicate residue with normal distribution and variance. ns = not significant.

Discussion

Seeds of *A. cearensis* support water content as low as 5.27% (LÚCIO et al., 2016) during storage, thus have an orthodox behaviour, being able to be stored for a long period in low temperatures and low relative humidity (GALÍNDEZ et al., 2015), with reduced respiratory rate (NASCIMENTO, 2009). The water content of *A. cearensis* seeds in this study was the same (9.2%) in all storage environments. Guedes et al. (2010a) found values from 6.25 to 15.89% in seeds kept in laboratory and 6.31 to 9.52% in seeds stored in refrigerator and they attribute the worst emergency values to the high water content.

Storage method at laboratory environment without container reduced FG and GR differing from fresh seeds and laboratory with airtight container respectively. Despite these differences in FG and GR, *A. cearensis* seed germination was higher than 70% and demonstrates the capacity of survival even with high oscillations of temperature and relative humidity (RH) that may have occurred on these storage conditions. Therefore, if variations in RH did not influence the loss of viability of the seeds in airtight container, it was probably the temperature oscillations that caused the germination percentage reduction. Seeds exposed to natural environment show decreasing viability over time (GUEDES et al., 2012b). These results related to oscillations in RH were sufficient to promote higher respiratory rates, leading to an increase in the consumption of seed reserves during respiration and accelerating the rate of deterioration. Therefore, both genetics characteristics as environmental conditions may contribute to the viability of storage seeds (CARVALHO et al., 2014).

The low FG of seeds stored in liquid N_2 after 24 months storage, could be attributed to the moment of withdrawal of the seeds from the N_2 flasks to refrigerator. It was observed that seeds had tissue disruption during thawing when were removed from liquid N_2 and this may have been one of the reasons for the low overall physiological quality of the seeds stored in ultra low temperatures. However, one hour dehydration on silica gel is still not sufficient for prolonged cryopreservation for *A. cearensis* seeds (Table 1).

Difference on frequency of germination by GU as of 12^{th} month from liquid N_2 seeds in relation to fresh seeds could be explain by the fact that low temperature of storage induced greater germination uniformity with potentially delayed values that suggest a capacity of wide spread germination over time over by a natural need of survival of the species (Table 3).

FE demonstrates to be a good test to qualify the deteriorated or low vigour seed by the fact that FE test is thinner (detectable) and easy to qualify the seed vigour of *A. cearensis*. FE also can be used to separate accessions aiming to use as reforestation and conservation for example. Many authors support the possibility of a relationship between emergence and seed vigour (DEMIR; MAVI, 2008; MILOŠEVIĆ et al., 2010 and PERVEEN et al., 2010) and *A. cearensis* seeds had high viability with 90% of germination at the 27th month for airtight container in laboratory and LN and present low emergence for this same period on field.

For ER gas exchange by the different storage conditions with and without container in laboratory did not prevent the increase of the emergency time (Table 5). Seed vigour is associated with deterioration process (SHELAR et al., 2008) and may have occurred the seed aging in seeds stored at laboratory out of airtight container influenced by temperature and by gas exchange. Different by SH that seed physiological quality in laboratory may be decreased only by temperature storage conditions.

The reserve mobilization in *A. cearensis* germinated seeds was directly influenced by the ultra-low temperatures in storage (Tables 7-8). These results evidenced the mobilization of the reserve compounds of the cotyledons (source) and their translocation to the root (drain) at low temperatures. Amadori and Maillard reactions explain decrease in RS content of seeds during storage (STRELEC et al., 2008). Amadori and Maillard reactions contribute to the deterioration of seeds (WETTLAUFER; LEOPOLD, 1991), stimulating respiration (LEPRINCE; VERTUCCI, 1995) and increasing the formation of free radicals (LEPRINCE et al., 1990). High levels of RS in *A. cearensis* roots at the last 3 storage period evaluated in LN can be lead to an interaction of glucose with free amino acids and subsequent membrane damage which may have led to seed tissue disruption (VESELOVA et al., 2015). Therefore, can be highlighted the selective mobilization of sugars during storage time at low temperatures and those levels could be attributed to stress factors.

Storing seeds in airtight containers at laboratory environment can maintain their quality for at least one year (Table 4). This allows maintainance of high seed vigour until seedling production in nurseries for reforestation in the next rainy season. Seeds should be kept well preserved at least until next season, that it is the period that normally occurs the flowering. Under laboratory environments, seeds typically lose their viability within months (BARBEDO et al., 2002.) and Caatinga seedlings can only be sowed at a very specific time (January to May) of soil water availability (BARBOSA et al., 2003; MEIADO et al., 2012). Therefore, these

studies, through the response of germinability time, allowed progress towards the understanding of seeds storage. And techniques regarding cryopreservation and the defrosting method should be improved for this species.

5.0 CHAPTER 2

SHALLOW PHYSICAL DORMANCY OF *Amburana cearensis* SEEDS AS ADAPTATION TO A SEMI-ARID ENVIRONMENT

ABSTRACT: We investigated seed imbibition and germination in a range of accessions and salt concentrations to understand how water uptake contributes to variation in stress response during early plant development. Accessions differed in seed dry mass, in time until 50% imbibition (IM*t50*), and time until radicle protrusion (RP). The start of water uptake (TWU) was delayed by more than 4d despite optimal contact between the seed surface and water, and this delay was stronger for smaller seeds and differed between accessions. Longer delay of imbibition was also correlated with higher optimum temperature for germination rate (T_o), and with longer time until radicle protrusion in water. The TWU, IM*t50*, and the RP differed between water and salt treatments for the accessions from the semi-arid habitat; in salt, seeds imbibe later, slower and take up more water prior to radicle protrusion. These results suggest that delayed water uptake portrays a form of shallow physical dormancy, and forms an adaptation to an environment with high temperature, low precipitation, and saline soils. This most likely spreads the risk of completing germination at the start of the rainy season, yet avoids too much restriction.

Keywords: Umburana, Caatinga, Fabaceae, imbibition phases, salt stress, seed germination

RESUMO: Foram investigadas a embebição e a germinação de sementes em acessos e concentrações de sal para entender como a absorção de água contribui para a variação na resposta ao estresse durante o desenvolvimento precoce da planta. Os acessos diferiram na massa seca da semente, no tempo até 50% de imbibição (IM*t50*) e no tempo até protrusão da radícula (RP). O início da absorção de água (TWU) foi atrasado em mais de 4d, apesar do contato ótimo entre a superfície da semente e a água, sendo maior para as sementes menores e diferiu entre os acessos. Um grande atraso na embebição também foi correlacionado com temperatura ótima mais alta para a taxa de germinação (*To*), e com tempo até a protrusão radícular em água. O TWU, IM*t50* e o RP diferiram entre os tratamentos de água e sal para as acessos do habitat semiárido; no sal, as sementes absorvem mais tarde, lentamente e ocupam mais água antes da protrusão da radícula. Estes resultados sugerem que a absorção tardia de água retrata uma forma de dormência física rasa e forma uma adaptação a um ambiente com alta temperatura, baixa precipitação e solos salinos. Isto provavelmente espalha o risco de completar a germinação no início da estação chuvosa, mas evita demasiada restrição.

Palavras-chave: Umburana, Caatinga, Fabaceae, fases de imbibição, estresse salino.

Introduction

Caatinga is the predominant native vegetation in the Northeast of Brazil (ALBUQUERQUE et al., 2007). Caatinga vegetation is found in a semi-arid region with a hot climate and low annual rainfall of 250 to 800 mm. The rainy season lasts 3 to 5 months and brings irregular and local rain, while the dry season lasts 7 to 9 months with virtually no rain and with strong, dry winds that contribute to the drought. The daily mean temperature reaches a maximum of 29.6 °C from October to January at the start of the rainy season, with common daily high temperatures of 37 °C (MAIA, 2008; MOREIRA et al., 2006 and REIS et al., 2012). The lowest daily mean temperatures are found in the months of June to August, when the daily average values are in the order of 24 °C (MANZI et al., 2006). Furthermore, salinity, sodicity or both simultaneously provide chemical, physical and biological changes in the soil (QADIR et al., 2007), which directly impacts on plant physiology including seed germination. Caatinga soils are rich in minerals, stony and with a low water retention capacity. Soil salinization occurs in areas where soils are shallow and water evaporation is fast due to heat, which forms a limiting factor for the production of crops in that region (ALVES et al., 2009).

Amburana cearensis (Arr. Cam.) A.C. Smith, populary known as “umburana-de-cheiro”, is a member of the Fabaceae family. It is a tree native to South-America, typical of the Caatinga biome and listed as endangered by the IUCN due to logging of larger trees and poor regeneration (IUCN, 2015). In Brazil it occurs from the Northeast to the South-west, and although predominantly growing in semi-arid environments it also shows good adaptation to the Atlantic rainforest. It is characterized as a deciduous tree in the Caatinga by the fall of the leaves during the dry season (LORENZI, 2008 and LORENZI; MATOS, 2002). The species shows variation in the time of flowering and fruiting between regions, leaving the seeds ready to germinate upon the first rains at the start of the rainy season (MAIA, 2008).

Successful plant development greatly depends on successful germination. Several environmental factors affect germination of seeds, in particular drought and osmotic stress (JAJARMI, 2009). High salinity levels inhibit germination via osmotic and/or toxic effects (SALI et al., 2015) and inadequate temperature inhibits by poor development of metabolic activities (OLIVEIRA et al., 2013). In addition to these factors, stresses as salinity, temperature and oxidation often cause cellular damage in Caatinga species, negatively affecting germination, plant growth and productivity with consequences for the morphology, physiology, biochemistry and molecular biology (DANTAS et al., 2015; DANTAS et al., 2014 and RIOS

et al., 2016). Germination requires water and the classical concept of seed imbibition is triphasic, with a rapid initial uptake of water (phase I) followed by a constant water content (phase II) prior to rupture of covering layers by the protruding embryo that concurs with a second increase in water uptake (phase III). However, phase II was recently demonstrated to consist of three sub-phases due to the increase in water uptake associated with testa rupture prior to phase III that is associated with endosperm rupture (TOOROP, 2015). The effect of salinity on the multiphasic imbibition curve was not studied. Germination of *A. cearensis* seeds was described as slow (VIEIRA et al., 2008), but imbibition was not studied.

In species of semi-arid environments, studies of physiological mechanisms that contribute to survival under drought, temperature and salinity stress have been extensively studied in cultivated plant species (DOGAN, 2009; KIRNAK; JAMIL et al., 2006 and JAJARMI, 2009). However, little is known about plant establishment and the mechanisms of adaptation of native species from the Caatinga under these conditions, including forest species as *A. cearensis*. This research investigates the influence of salt stress on seed imbibition and germination of *A. cearensis*, thus generating knowledge that supports regeneration of this endangered and economic species.

Materials and Methods

Plant material

Seeds of *A. cearensis* were collected from dehiscent fruits, from eight individual trees (accessions, named by letters) in September 2014 in Lagoa Grande PE (accessions A to G; S 8º34’04,00’’; O 040º10’18,00’’) and Jacareí SP (accession H; S 23º17’49,30’’; O 045º58’05,00’’; Figure 4). The seed weight was determined weighing individual seeds in samples taken from each accession (10 seeds and 3 replicates). Seed material was kept at 15 °C and 15% RH until experiments were conducted.

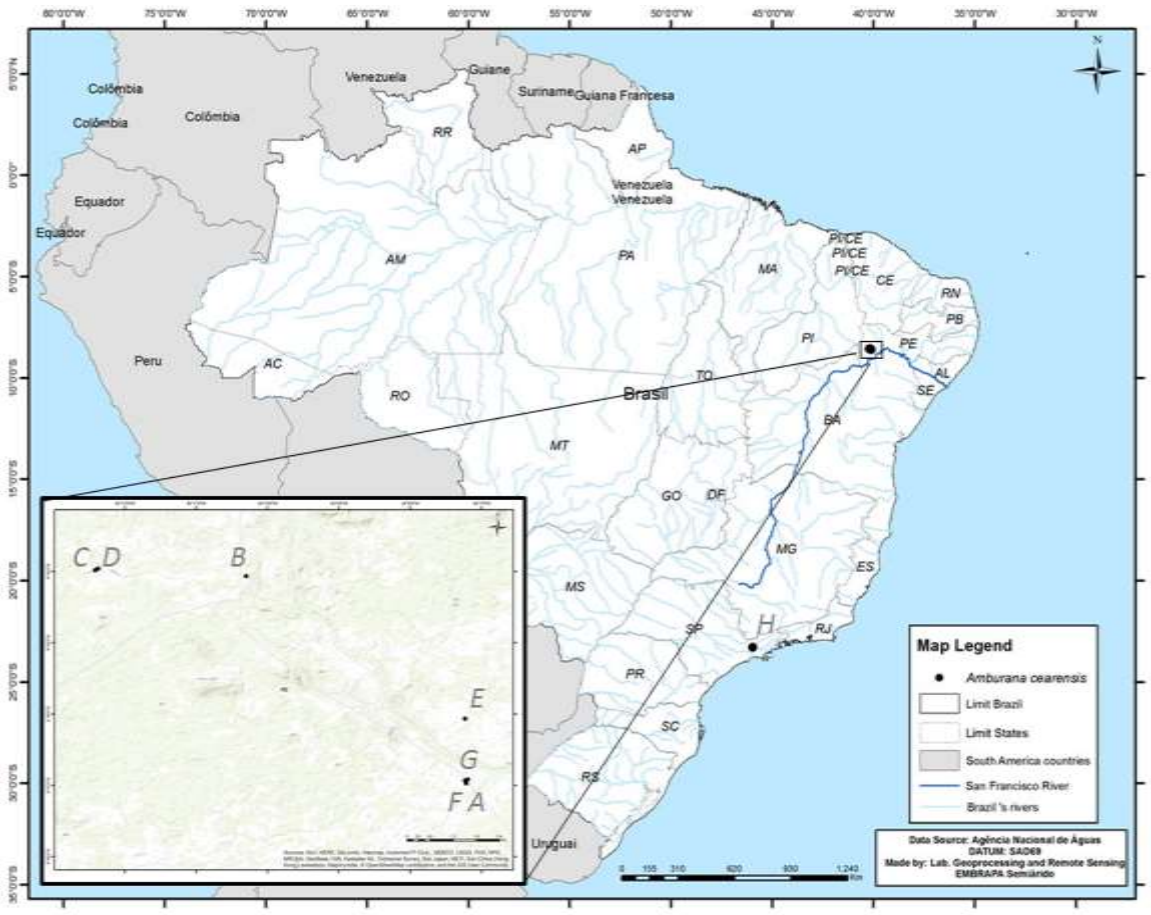


Figure 4. Map of the collecting area of each accession (A to H) of *A. cearensis* in Lagoa Grande / Pernambuco state (North dots) and Jacareí / São Paulo state (South dot) in Brazil. (Made by: Lab Geoprocessing and remote sensing EMBRAPA Semiárido)

Germination

Three replicates of 25 seeds for each treatment were sown in germination boxes of 15 x 10 x 5 cm filled with 50 ml of solution gelled with sterile agar 1%, Figure 2L. The boxes were wrapped in transparent plastic zip-lock bags, in order to prevent loss of water by evaporation.

The germination tests were performed in temperature-controlled incubators (Figure 2J and K) at the following temperatures: 15, 20, 25, 30, 35, 40 and 45 ºC with a photoperiod of 12 hours for all accessions. Seed germination scoring was performed twice a day. Germination was considered as complete when tegument rupture was observed with radicles protruding more than 1 mm. The results for final germination (FG) were expressed as a percentage and time to half-maximal germination (G*t50*, h) was calculated after sigmoidal curve fitting using the Boltzmann equation (TOOROP et al., 2012).

Germination rate (GR) was calculated as the reciprocal of the time to half-maximal germination (1/G*t50*, h^{-1}) and was plotted as a function of temperature and regressed using a linear model, to estimate the base temperature (T_b), optimum temperature (T_o) and ceiling temperature (T_c) for each population (GARCIA-HUIDOBRO et al., 1982). From the germination data uniformity (GU) was calculated as time elapsed from 20% to 80% germination.

To test the response to salinity, three replicates of 25 seeds were submitted to germination in rolls of two layers of filter paper (Whatmann no.1) wetted with 2.5 times the weight of the paper in moisture with saline solution, using 0, 100, 200, 300, 400 and 500 mM of NaCl. Seeds were incubated at 38 °C with a photoperiod of 12h. Germination was scored twice a day.

Imbibition and seed dimensions

Three replicates of 10 seeds were used for each accession and seeds were weighed individually dry and subsequently during imbibition every three hours until radicle protrusion (Figure 2K). Seeds were placed in rolls of two layers of filter paper, moistened with distilled water or a 300mM NaCl solution using 2.5 times the weight of the paper. Paper rolls were placed in plastic zip-lock bags and transferred to an incubator at 38 °C with a photoperiod of 12h.

Water was added to the initial weight to correct for any evaporation. The seed weight of individual seeds was taken on a 4-place balance.

Prior to imbibition on water, twenty seeds of each accession were used to measure the length, width and thickness of seeds using a calliper.

Distance to the nearest river was measured by maps made by Laboratory of Geoprocessing and remote sensing - EMBRAPA Semiárido.

Other parameters that were determined on individual seeds: the seed dry weight (DW, g), the final fresh weight upon imbibition prior to radicle protrusion (FW, g), the time until 50% imbibition or the time until 50% weight increase prior to radicle protrusion (IM*t50*), the imbibition uniformity or the time from 20 to 80% of the weight increase prior to radicle protrusion (IMU), the time until radicle protrusion (RP), and the time until the start of water uptake (TWU) calculated as the intersect between the linear regression lines of the initial weight

that remained constant for 4 days and the steady weight increase that characterised the start of phase I of water uptake.

Data analysis

Data were analysed using the Kruskal–Wallis analysis of variance to test differences between treatments. Principle Component Analysis (PCA) was performed using the parameters DW, FW, IM*t50*, IMU, RP, TWU in both water and 300 mM NaCl, supplemented with T_b, T_o, T_c, length, width, thickness, and distance of the maternal tree to the nearest river. Spearman's rank correlations were calculated for the same traits among accessions. The Mann–Whitney U test was applied for post-hoc comparison of sets of two treatments within each group. All tests were performed in GenStat (v.14.2; http://www.vsni.co.uk/).

Results

Germination

Seed germination reached 100% between 20 and 40 ºC. With 76% at 15 ºC and 0% at 45 °C germination was lower at these extremes than at all other tested temperatures (H = 31.26, P < 0.001; Figure 5A). No recovery of germination was observed upon transfer of un-germinated seeds, imbibed and incubated at 45 ºC, to 38 °C. In contrast, all seeds incubated at 15 ºC and subsequently transferred reached 100% germination (data not shown).

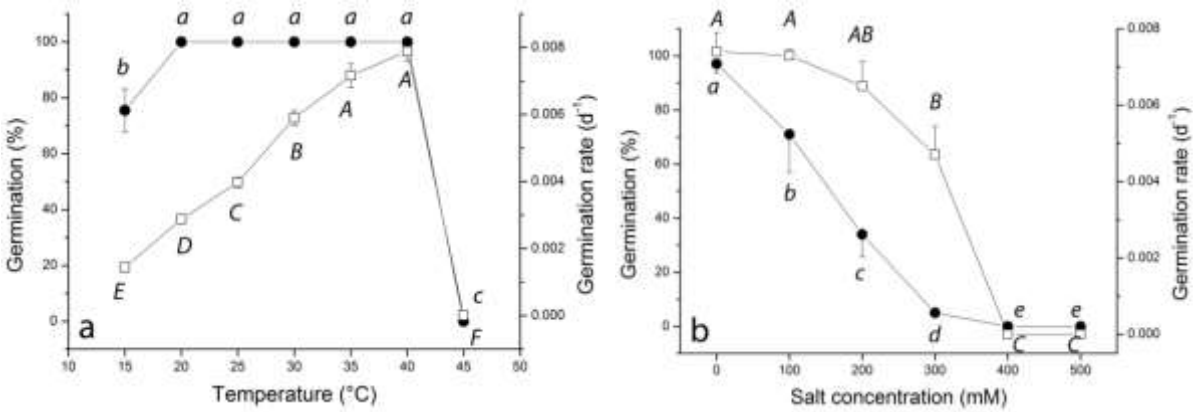


Figure 5 Final germination (closed circles) and germination rates (open squares) of *Amburana cearensis* seeds at a range of constant temperatures (A), and in a range of NaCl solutions at 38 °C (B). Values with the same letters (capitals for rate, small font for germination) do not differ significantly at P < 0.05. Data are means, error bars represent standard error of the mean, n = 8.

The mean GR increased steadily between 15 and 40 °C (Figure 5A). The cardinal temperatures were calculated in all accessions separately and mean values were 10 °C for T_b and 38 °C for T_o. The GU in water improved with an increasing temperature (not shown).

FG of *A. cearensis* seeds differed between the applied salt concentrations ($H = 88.51$, $P < 0.001$), decreasing with an increase in NaCl concentration. Although germination in 300 mM NaCL was 5% for the accession in this experiment, this differed widely between accessions (not shown). No germination was observed at 400 mM or higher (Figure 5B). The GR declined above 200 mM (Figure 5B).

Water uptake

Despite being harvested from dehiscent mature fruits, seeds differed in dry weight ($H = 108.1$, $P < 0.001$), ranging from 0.38 g in accession B to 0.66 g in accession H (Figure 6).

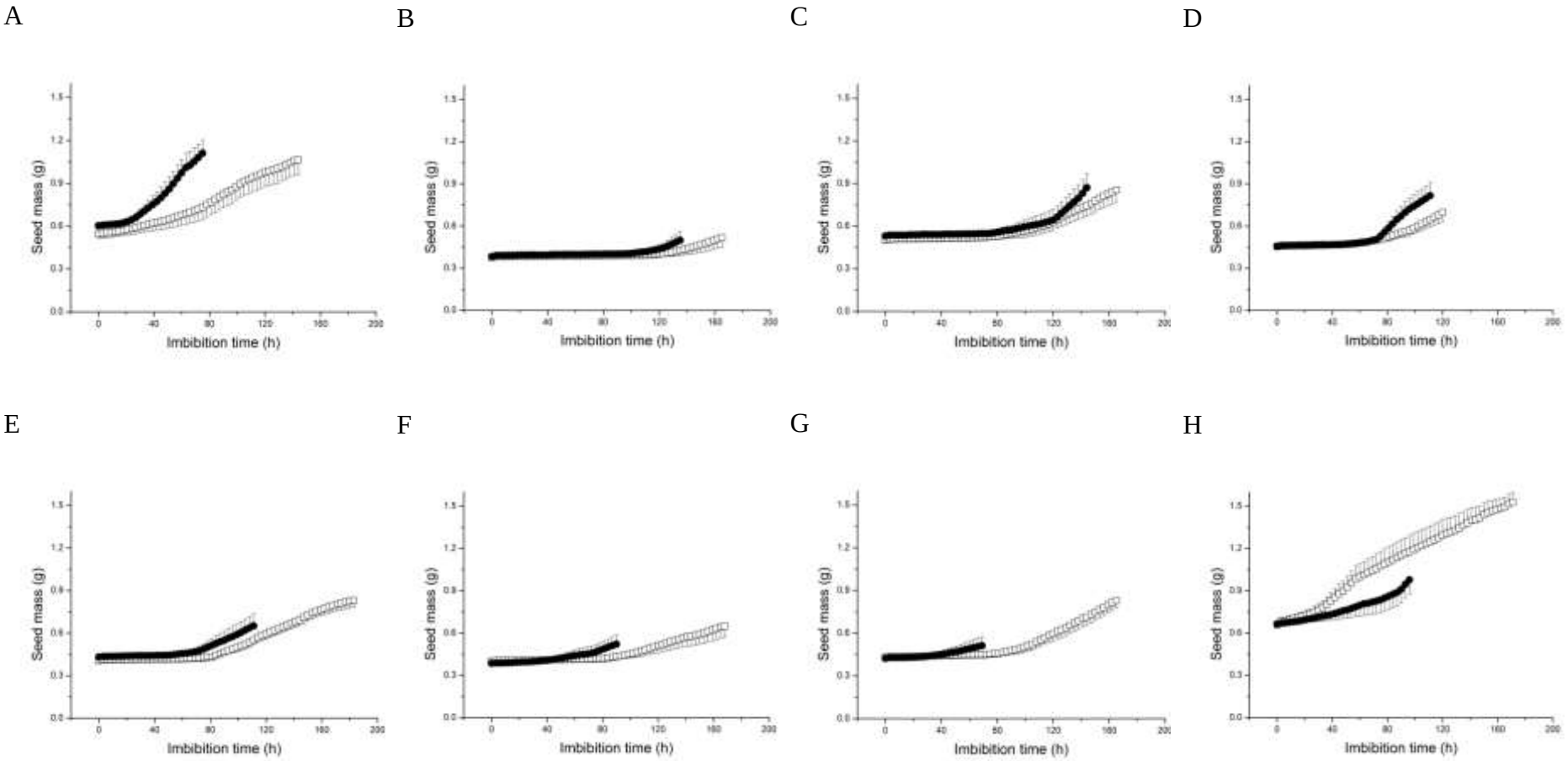


Figure 6. Imbibition curves of each accession (A to H) of *A. cearensis* seeds in water (closed circles) and 300 mM NaCl (open squares). Data are means, error bars represent standard error of the mean, n = 10.

Imbibition curves of *A. cearensis* seeds in water and in 300 mM NaCl did not follow the classical triphasic pattern. Instead, a phase zero was observed that varied in duration from 1.9 d in accession A to 6.4 d in accession B (H = 64.97, P < 0.001), characterised by absent increase in weight before entering phase I. Imbibition on 300 mM salt solution appeared to result in 10% longer time until water uptake (TWU) in most of the accessions (Figure 6). IM*t50* differed between the accessions (H = 53.43, P < 0.001) and between the water and salt treatments (H = 7.27, P = 0.007), resulting in a 19% higher value in salt. Time of radicle protrusion RP differed between the accessions (H = 23.20, P = 0.002) and between the water and salt treatments (H = 30.09, P < 0.001), resulting in a 27% higher value in salt. The bigger difference for IM*t50* than TWU seems to be associated with a higher increase in water content in salt, since FW at the time of RP, but not DW, differed between the water and salt treatment (H = 4.52, P = 0.034).

The PCA showed clustering of accessions A, D, E, F and G. The first component of the PCA explained 58% and the second component 19% of the variance (Figure 7). The latent vectors showed a similar direction for TWU, IM*t50* and RP, which explained more of the first two components than any other factor. Almost perpendicular to these were the analogous parameters for the salt treatment, TWUs, IM*t50*s, and RPs, with a similar direction as T_o (Figure 7). Accessions A, D and H had the lowest T_o with 35.9, 35.8 and 35.5 ºC, respectively. The highest T_o was observed for accession B with 39.4 ºC (Figure 7). False-colour coding for T_o of accessions in the PCA explained most of the first component. Length and width of seeds explained more of the variance than thickness (Figure 7) and showed better correlations with the seed mass DW (Table 9). Of the three seed dimensions, length and width were correlated with T_o, but not thickness (Table 9).

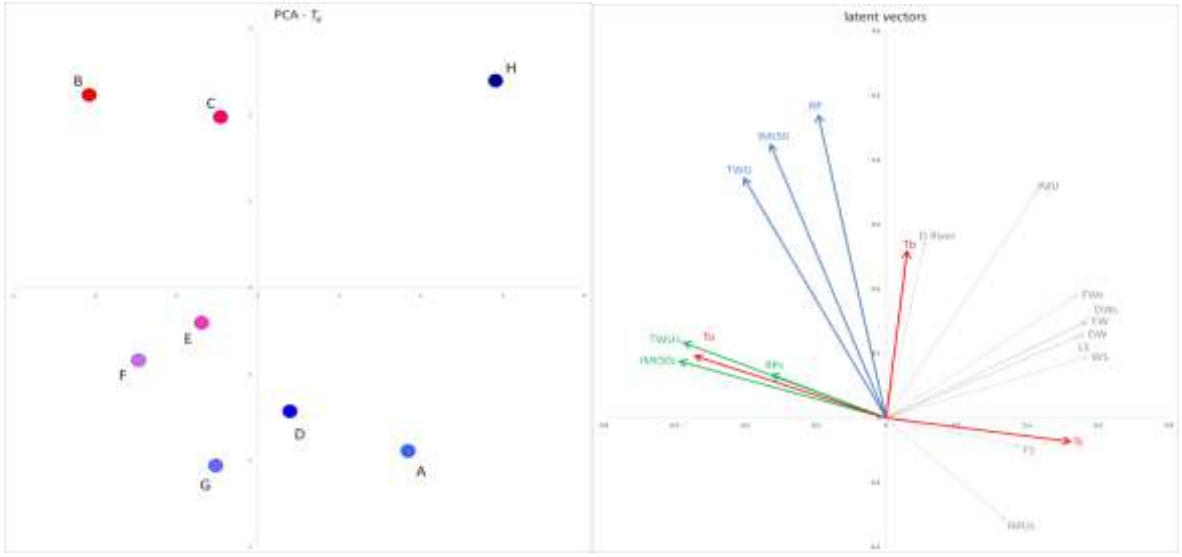


Figure 7. Principal component analysis with 8 accessions showing component 1 that explains 51% of the variance and component 2 that explains 18% of the variance (A), and latent vectors (loadings; B) with the following traits of *A. cearensis* seeds. DW, seed dry weight; FW, seed fresh weight at time of radicle protrusion; IM*t50*, time until 50% imbibition; IMU, imbibition uniformity; RP, time until radicle protrusion; TWU, time until water uptake; T_b, cardinal base temperature; T_o, cardinal optimal temperature; T_c, cardinal ceiling temperature; D River, distance to the nearest river; LS, seed length; WS, seed width; TS, seed thickness. The suffix s denotes the same parameter in 300 mM Nacl as in water.

Correlation analysis showed that seed mass was not correlated with imbibition speed in water (IM*t50*) nor with the length of phase 0 (TWU); however, seed mass was correlated with these parameters in the salt treatment (IM*t50*s and TWUs; Table 9; Figure 8), suggesting that in a salt solution water uptake was more strongly delayed in smaller seeds, and these entered phase I of the imbibition curve later. Larger seeds had poorer imbibition uniformities in water but not in salt solution (Table 9). Accessions with larger seeds also had a lower optimum temperature for germination (Table 9).

A B

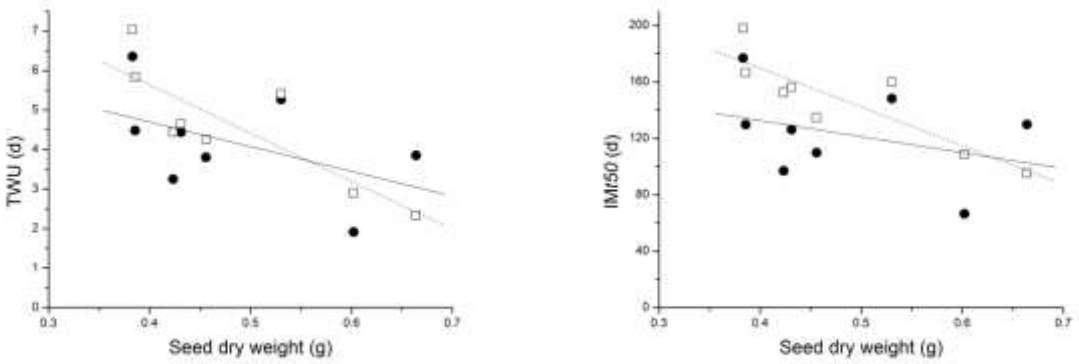


Figure 8. Scatter plots for the seed dry weight versus the time until water uptake (A) or the imbibition speed (B) in water (closed circles) and 300 mM NaCl (open squares). Data are means, n = 10, lines are linear regression for water (solid) and NaCl (dashed) treatments.

Optimum germination temperature T_o was higher for accessions with longer time in water prior to radicle protrusion, IM*t50* and TWU, as well as in salt solution, IM*t50*s and TWUs (Table 9; Figure 9). Consequently, the time until radicle protrusion was also longer in water and in salt for accessions with higher T_o (Table 9). Shorter time to imbibe correlated with shorter time to RP in water and in salt, both through TWU and IM*t50* (Table 9; Figure 10).

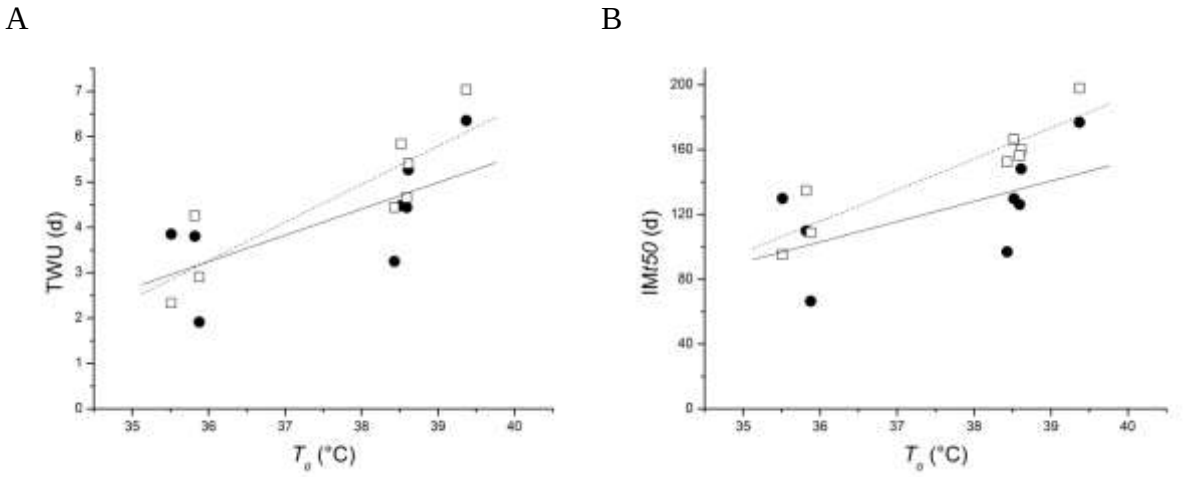


Figure 9. Scatter plots for the cardinal optimal temperature (T_o) versus the time until water uptake (A) or the imbibition speed (B) in water (closed circles) and 300 mM NaCl (open squares). Data are means, n = 10, lines are linear regression for water (solid) and NaCl (dashed) treatments.

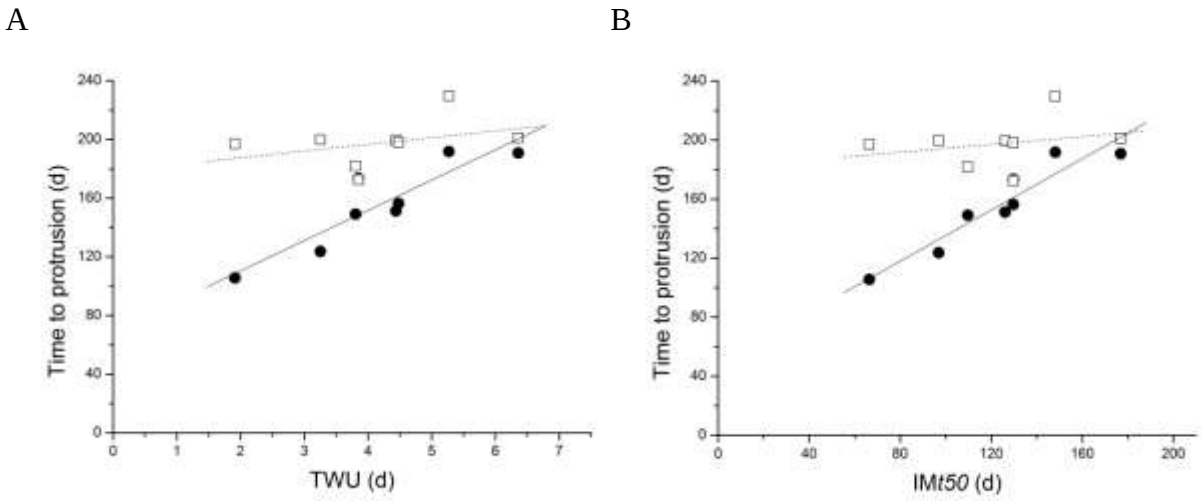


Figure 10. Scatter plots for the time until water uptake (A) or the imbibition speed (B) versus the time to radicle protrusion in water (closed circles) and 300 mM NaCl (open squares). Data are means, n = 10, lines are linear regression for water (solid) and NaCl (dashed) treatments.

Spearman's rank correlation coefficient																				
		DW	FW	IMt50	IMU	RP	TWU	DWs	FWs	IMt50s	IMUs	RPs	TWUs	Tb	To	Tc	D_River	LS	WS	TS	
Probabilities		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
DW	1	*	1	-0.238	0.714	-0.119	-0.476	0.976	0.857	-0.833	0.381	-0.524	-0.833	0.19	-0.643	0.571	-0.143	0.905	0.976	0.643	1
FW	2	0	*	-0.238	0.714	-0.119	-0.476	0.976	0.857	-0.833	0.381	-0.524	-0.833	0.19	-0.643	0.571	-0.143	0.905	0.976	0.643	2
IMt50	3	0.134	0.134	*	0.333	0.976	0.929	-0.286	-0.167	0.571	-0.619	0.405	0.571	0.405	0.548	-0.333	0.381	-0.357	-0.286	-0.405	3
IMU	4	0.011	0.011	0.097	*	0.405	0.119	0.619	0.452	-0.381	0.19	-0.071	-0.381	0.643	-0.071	0.286	0.286	0.405	0.619	0.548	4
RP	5	0.188	0.188	0	0.075	*	0.905	-0.167	-0.071	0.524	-0.571	0.429	0.524	0.429	0.524	-0.381	0.262	-0.262	-0.167	-0.381	5
TWU	6	0.054	0.054	0	0.188	0.001	*	-0.548	-0.452	0.81	-0.69	0.571	0.81	0.357	0.762	-0.619	0.238	-0.619	-0.548	-0.452	6
DWs	7	0	0	0.115	0.024	0.166	0.038	*	0.929	-0.857	0.405	-0.5	-0.857	0.143	-0.69	0.619	-0.167	0.952	1	0.524	7
FWs	8	0.002	0.002	0.166	0.061	0.21	0.061	0	*	-0.738	0.143	-0.476	-0.738	0.095	-0.667	0.595	-0.19	0.952	0.929	0.286	8
IMt50s	9	0.003	0.003	0.033	0.082	0.043	0.004	0.002	0.009	*	-0.643	0.762	1	0.19	0.905	-0.833	0.024	-0.881	-0.857	-0.595	9
IMUs	10	0.082	0.082	0.024	0.155	0.033	0.014	0.075	0.176	0.021	*	-0.262	-0.643	-0.19	-0.476	0.405	0.19	0.31	0.405	0.333	10
RPs	11	0.043	0.043	0.075	0.21	0.067	0.033	0.049	0.054	0.007	0.125	*	0.762	0.571	0.905	-0.69	-0.048	-0.667	-0.5	-0.405	11
TWUs	12	0.003	0.003	0.033	0.082	0.043	0.004	0.002	0.009	0	0.021	0.007	*	0.19	0.905	-0.833	0.024	-0.881	-0.857	-0.595	12
Tb	13	0.155	0.155	0.075	0.021	0.067	0.09	0.176	0.198	0.155	0.155	0.033	0.155	*	0.548	-0.119	0.095	-0.095	0.143	0.31	13
To	14	0.021	0.021	0.038	0.21	0.043	0.007	0.014	0.017	0.001	0.054	0.001	0.001	0.038	*	-0.786	0.071	-0.833	-0.69	-0.333	14
Tc	15	0.033	0.033	0.097	0.115	0.082	0.024	0.024	0.029	0.003	0.075	0.014	0.003	0.188	0.005	*	0.143	0.69	0.619	0.476	15
D_River	16	0.176	0.176	0.082	0.115	0.125	0.134	0.166	0.155	0.234	0.155	0.22	0.234	0.198	0.21	0.176	*	-0.238	-0.167	-0.167	16
LS	17	0.001	0.001	0.09	0.075	0.125	0.024	0	0	0.001	0.107	0.017	0.001	0.198	0.003	0.014	0.134	*	0.952	0.429	17
WS	18	0	0	0.115	0.024	0.166	0.038	0	0	0.002	0.075	0.049	0.002	0.176	0.014	0.024	0.166	0	*	0.524	18
TS	19	0.021	0.021	0.075	0.038	0.082	0.061	0.043	0.115	0.029	0.097	0.075	0.029	0.107	0.097	0.054	0.166	0.067	0.043	*	19
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	*

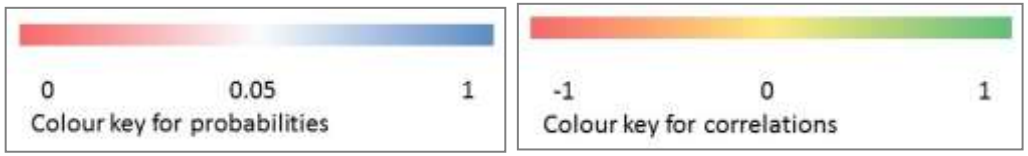


Table 9. Spearman's rank correlation coefficient matrix of *Amburana cearensis* **DW** - Dry weight; **FW** - Final weight; **IM*t50*** - Time until 50% imbibition; **IMU** - Imbibition uniformity; **RP** - Time until radicular protrusion; **TWU** - Time until water uptake which is the intersect; **DWs** - Dry weight in salt solution; **FWs** - Final fresh weight in salt solution; **IM*t50*s** - Time until 50% imbibition in salt solution; **IMUs** - Imbibition uniformity in salt solution; **RPs** - Time until radicular protrusion in salt solution; **TWUs** - Time until water uptake which is the intersect in salt solution; T_b - Base temperature; T_o - Optimum temperature; T_c - Ceiling temperature; **D_River** - Distance to the nearest river. **LS** - Length of seeds; **WS** - Width of seeds; **TS** - Thickness of seeds.

Discussion

The mean optimum temperature for germination speed of *A. cearensis* seeds was 38 ºC, similar to results by Guedes et al. (2010c), which to date is the highest T_o reported for any tree species (DÜRR et al., 2015). Seed mass, mainly due to seed length and width, differed among the eight accessions and mass was correlated negatively with T_o. Seed mass was also associated with time until first water uptake, and with time until 50% imbibition in salt solution. Consequently, differences in T_o were associated with the time until water uptake and the speed of water uptake, both in water and in salt solution. The strong association with T_o, rather than T_b, is sensible for a species in an environment where temperatures show hardly any seasonal variation throughout the year. These results suggest that intraspecific variation in seed mass and in imbibition patterns assist in spreading the risk of starting the life cycle in this species.

Seeds of *A. cearensis* have been described to not contain any form of dormancy, including physical dormancy (BASKIN; BASKIN, 2014). Although our data confirmed this, imbibition was delayed and this delay was stronger in some accessions than in others. Delayed imbibition was also described by Loureiro et al. (2013), who found that a macrosclereid structure impedes water uptake and scarification overcomes it. Imbibition delay was characterised by an additional phase zero (or TWU) to the classical triphasic imbibition curve, not usually observed in non-dormant seeds, which formed an intermediate phenotype between physical dormancy and uninhibited imbibition. This phase zero functionally mimicked the loss of physical dormancy in a short time span and was longer for smaller seeds. *A cearensis* is a member of the Fabaceae and physical dormancy occurs with high frequency in this family. According to Meisert (2002) the extent of physical dormancy or hardseededness mainly occurs in arid habitats or semi-arid. Seed dormancy has both advantages and disadvantages for plants; weak dormancy leads to more uniform germination with reduced spreading over time leading to higher fitness but at a greater risk, whereas strong dormancy inhibits germination within a limited period, resulting in reduced fitness but with a reduced risk (CHILDS et al., 2010; FENNER, 2012). Delayed imbibition can be interpreted as an adaptation to an environment where stochasticity of precipitation is high at the start of the short rainy season and physical dormancy can be too stringent a condition to form a benefit (MARTINS et al., 2015).

As a result of the correlation with higher optimum temperature, these seeds were also subject to a higher risk of deterioration (KAPOOR et al., 2011). The longer phase zero can be explained as a mechanism that delays water uptake to protect against the deteriorative effect of

high temperatures, while still allowing imbibition albeit with delay to avoid missing the right time of year to germinate. The latter is relevant in a habitat with very little and highly seasonal precipitation. Very slow germination in soil was reported for *A. cearansis*, which is supported by our laboratory observations, resulting in completion of germination later in the rainy season that has erratic patterns of rainfall (VIEIRA et al., 2008). This conservative strategy spreads the risk of mortality, in contrast with the rapid germination of another arboreal species that occurs in the Caatinga, *Handroanthus impetiginosus*, but with different adaptation in the form of post-germination desiccation tolerance (MARTINS et al., 2015). The germination uniformity was correlated negatively with seed size, and poorer uniformity was displayed by smaller seeds. This concurs with the longer phase zero of imbibition (TWU), spreading the risk of completing germination when seeds are more likely to deteriorate due to higher temperatures. Together, the results suggest that small seed size, poor germination uniformity and prolonged phase zero formed an adaptive mechanism to the high temperature and low precipitation in a semi-arid environment.

In summary, *A. cearensis* showed adaptation to an environment with a constant high temperature and low erratic precipitation, with an optimum temperature for germination speed of 38 ºC, yet with a risk spreading strategy through variation in seed dimensions and seed weight associated with delayed imbibition. Associated lack of imbibition uniformity and germination uniformity further contributed to this strategy, thus compensating for the very broad optimum temperature for final germination and functional lack of dormancy.

6.0 CONCLUDING REMARKS

The use of native tree species for reforestation programs or urban forestation has intensified in recent years, and many species have high regeneration capacity in degraded areas, as well as the ability to develop under wide range in water restriction and temperatures with high germination rates, which attributes to this species a great ability to regenerate the Caatinga biome. Thus pioneer and drought tolerant species, such as *A. cearensis*, have great ecological importance in recomposing the landscape of degraded Caatinga areas.

A good storage method is essential for maintaining seed viability. In the Caatinga biome, storage becomes a fundamental tool to ensure annual demand of seeds, allowing the seed storage *ex situ* in years of low production as a strategy to ensure the preservation of the species and quality of seeds for sowing. *A. cearensis* seeds presented high vigour when stored in a refrigerator for at least 27 months, which guarantees its conservation and the planning of recovery of degraded areas.

The optimum germination temperature for *A. cearensis* seeds is 38 ºC. This optimum temperature is below to the average maximum air temperature in the Caatinga climate (MANZI, 2006.) and demonstrates that this species is adapted to warm environments favouring the high germinability rates.

On the other hand, *A. cearensis* seeds were affected by increases in salinity levels and no germination were observed above 300 mM (approximately -0.75 MPa and 19 $dS.m^{-1}$). According to Valladares et al. (2004), soils characterized by electrical conductivity from 4 $dS.m^{-1}$ are considered saline. In this case *A. cearensis* seeds show to be resistant to saline soils.

The imbibition curve presents a different model and showed long phase zero with differences between accessions and shows an intermediate phenotype between physical dormancy and uninhibited imbibition. Furthermore, variation in seed dimensions and seed weight were associated with delayed imbibition as strategy to spreading the risk along the time to ensure the survival of the specie.

Based on the above considerations, it is evident the importance of conservation and physiological factors studies that will minimize the impacts on species threatened with extinction and *A. cearensis* shows good adaptation to harsh environments and have an important role in the recovery of degraded areas.

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