Physiological and morphological aspects of seed viability of a neotropical savannah tree, *Eugenia dysenterica* DC.

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Summary

The morphology of *Eugenia dysenterica* DC. (Myrtaceae) seeds and post-seminal development was studied and its germination response investigated in relation to temperature, desiccation under different drying regimes and storage under three temperatures. The seeds are elliptical, varying from globose to half-globose and seedlings are cryptogeal with long and slender axial roots. High germination percentages were achieved at temperatures ranging from 15° to 30°C and the thermal optimum was about 24°C. Seeds presented high moisture contents on shedding (47-53%) and completely lost viability when moisture contents were reduced to values below 18-22%; a straight-line relationship was observed between the probit of germination and moisture content percentage. These characteristics provide evidence of a recalcitrant storage behaviour of this species. Storage at near 45% moisture content enabled seed survival for 175 days with over 50% viability, in spite the storage temperature.

Introduction

Seeds of some tropical tree species have elevated moisture contents on shedding, tolerate very little subsequent dehydration and are often chilling-sensitive (Chin and Roberts, 1980). Such seeds have been called recalcitrant by Roberts (1973), in contrast to orthodox seeds that can tolerate desiccation without damage and can be safely stored at low temperatures and with low moisture contents (less than 5%). Furthermore, recalcitrant seeds are metabolically active and, in general, will initiate germination upon or shortly after shedding and stored seeds will deteriorate more or less rapidly, even in wet medium and at warm temperatures (Chin and Roberts, 1980; Farrant, Pammenter and Berjak, 1988; Berjak, Farrant, Pammenter, 1989). More recently, a third category of seed storage behaviour intermediate between orthodox and recalcitrant has been introduced (Ellis, Hong and Roberts 1990; Ellis, Hong, Roberts and Soetisna, 1991). Intermediate seeds are relatively desiccation-tolerant, but will not withstand removal of water to levels as low as orthodox seeds. These species, particularly if they are of tropical origin, may also be chilling-sensitive, even in the desiccated state (Hong and Ellis, 1996; 1998). Knowledge of seed storage behaviour is thus a pre-requisite for "ex situ" plant biodiversity conservation by seed storage (Hong and Ellis, 1998).
Because of their wide geographical distribution and the diverse ecological requirements of its species, seed storage behaviour in Myrtaceae is also diverse, comprising at least two of the three possible categories e.g., *Eucalyptus* spp. and *Psidium guajava* are orthodox (Hong, Linington and Ellis, 1996) and *Myrciaria cauliflora* is recalcitrant (Valio and Ferreira, 1992). *Eugenia dysenterica* DC. (Myrtaceae) is a tropical fruit tree endemic to a narrow range in the cerrado vegetation of central Brazil. Its edible fruits are commonly used by local populations, consumed *in natura* or used to make juice or fruit-jelly (Farias Neto, Fonseca, Gomide and Silva, 1991). A previous study of *E. dysenterica* seed storage (Farias Neto et al., 1991) reported cold storage (10°C) and plastic bags as the best methods of “ex situ” seed conservation.

Among the most threatened ecosystems occupied by the Myrtaceae is the neotropical savannah complex. The climatic pressure (especially the drought stress) exerted on plants growing in savannah-like ecosystems is accompanied by a gradually increasing human impact expressed either directly (e.g. land management) or indirectly (e.g. fires, overgrazing) (Ratter, Ribeiro and Brigdewater, 1997). Therefore, investigation of the mechanisms concerning seed germination, seedling establishment and seed conservation are of great importance to both conservation and regeneration, natural or artificial, of the savannah tree ecosystems. Additionally, of the numerous published papers on seed germination and seedling growth and survival, only a handful describe the initial morphology, size, or developmental stage of the seedlings studied, which are essential for understanding ecological processes (Garwood, 1996) and correct interpretation of germination tests of stored seeds (Oliveira, 1993).

The objective of this study was to provide recommendations for short-term seed storage of *Eugenia dysenterica*, based on the description of the morphology of seeds and post-seminal development, on the identification of the optimum temperature for seed germination, on the characterisation of the seed storage behaviour and on the study of seed storage under different temperatures during 350 days.

**Materials and methods**

**General**

Mature fruits of *E. dysenterica* were hand-collected in 1995, from nine trees of the cerrado vegetation, near the Brasília National Park (15°40’S, 47°58’W, 900 m above sea level in central Brazil). Fruits were mixed and immediately enclosed in heavy plastic bags before transportation to the Laboratory of Genetic Resources Conservation, EMBRAPA/CENARGEN, in Brasília. Fruits were considered mature when completely yellow coloured. Seeds were immediately extracted from the fruits (pericarps removed by washing in tapwater), cleaned and surface sterilised with sodium hypochlorite (1% v/v) for 10 minutes and surface dried for 30 minutes under laboratory conditions to remove water excess. Immature, rotten and insect-infested seeds were removed by visual inspection. Initial moisture content and germination were 48-52% and 94-98%, respectively. Experiments were conducted as soon after seed collection as possible, generally within 1-2 days. During this time seeds were kept at 15°C enclosed in plastic bags.
Desiccation and storage of Eugenia dysenterica seeds

Seed and seedling morphology
For post-seminal development descriptions, three sets of 15 seeds were sown in transparent plastic boxes filled with vermiculite and kept under laboratory conditions (27-31°C ambient temperature and 80% relative humidity, RH), on the window-sill, in order to receive 3-5 hours per day of direct sunlight. Descriptive terminology was based on Stearn (1992); seeds and seedlings were drawn with the aid of a camera lucida fitted on a stereomicroscope.

Germination at constant temperature regimes
Germination tests were performed in transparent plastic boxes (12 × 12 × 4 cm) on moist, heat-sterilised vermiculite on four replicates of 25 seeds per treatment, at temperatures in 5°C increments from 5° to 40°C and with 8-hour daylengths (photon flux density approx. 90 µmol m⁻² s⁻¹ from cool white fluorescent light sources). The seed coat was chipped to facilitate water and oxygen uptake, through partial removal of coat (window), according to Rizzini (1970). Germination was assessed at regular intervals over a period of approximately 12 weeks and re-wetted when necessary. Germinated seeds were counted and removed. The criterion for germination was >5-mm-long radicle protrusion. The rate of seed germination was estimated using the index of germination velocity (IGV) (Labouriau, 1970): 

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IGV = \frac{\Sigma n_i}{\Sigma n_i \cdot t_i}, \quad \text{where } n_i \text{ is the number of seeds germinated between } t_{i-1} \text{ and } t_i \text{ and } t_i \text{ is the number of days between the beginning of the experiment and the } i^{th} \text{ observation.}
\]

Desiccation studies
Two desiccation methods were employed: (a) seeds were desiccated in a monogranular layer on a suspended plastic mesh in a 15% RH and 24°C chamber and, (b) seeds were kept at 15°C in 6-L plastic desiccators with 2 L of silica gel (20% RH). The silica gel to seed ratio was 2:1 (w/w) and silica gel was regularly regenerated when it began to lose the deep blue colour of the indicator. Air inside the room was stirred constantly by a small fan. All desiccators were aerated at least weekly. The relative humidity of the air inside small, closed desiccators was assessed with a small hygrometer after 1-3 days of seed equilibration.

Seeds were removed from drying after different periods, depending on the rates of drying, to establish a wide range of seed moisture contents. Samples of 120-160 seeds were drawn when desirable moisture contents were achieved, according to the equation described in Cromarty, Ellis and Roberts (1982). Control samples were drawn at the beginning of the treatments.

Before sowing, dried seeds were re-hydrated to about 40-45% moisture contents by equilibration for periods up to 7 days in a monogranular layer on a suspended plastic mesh inside plastic desiccators with distilled water (95-98% RH and 24°C). For the equilibrium moisture content/relative humidity isotherm study, embryos (seeds without coat) were incubated in solutions of polyethylene glycol (PEG) at 10°C. Five 15-cm diameter Petri dishes per concentration were used, each containing 25 ml of autoclaved solution and 5 embryos. PEG solutions were changed after three and five weeks; embryos were rinsed, surface-dried and weighed weekly until a constant weight was reached (approx. 7 weeks),
when moisture content were determined. PEG Solutions were prepared according Michel and Kaufmann (1973) and the water potential $\Psi_w$ (MPa) of the PEG solutions was calculated according Nobel (1970). The solute potential was considered identical to water potential.

**Storage methods**

Seeds treated with Benlate (5% w/w active ingredient, benomyl) were packed in micro perforated folded-over 20$\mu$m gauge polythene bags and stored in chambers at 15, 10 and 5°C (± 0.5°C). The storage treatments comprised a factorial combination of the three temperatures and eight periods (0, 50, 100, 150, 200, 250, 300 and 350 days). At each storage interval, 140 seeds were removed at random for germination and moisture content determination. Seeds that germinated during storage were considered as viable if they were capable of producing a long radicle.

Germination tests for the experiments on desiccation studies and storage methods were made as described previously, but at 25°C. Moisture contents were determined on four replicates of ten seeds, by drying at 103 ± 2°C for a period of 17 hours (ISTA, 1999). All moisture contents given were based on wet mass basis.

**Results and discussion**

**Seed and seedling morphology**

The mature fruit of *E. dysenterica* (17.4 ± 2.9 g; n = 50) is a yellow globose bacca (berry; sensu Spjut 1994), with a fleshy mesocarp containing 1 – 4 seeds. Seeds (0.9 ± 0.3 g; n = 50) are irregularly elliptical, varying from globose to half-globose, flattened at one side. Circular or elliptical in outline with round borders (figure 1a, b), seeds have a thin (~1 mm), double-layered seed coat (figure 1d). The outer surface is comprised of a fibrous, easily detachable layer that confers a whitish colour to the dry seed. The inner seed coat consisted of a papery, deep-brown layer firmly attached to the seed; seminal stigmas are represented by a conspicuous yellowish raphe that extends along the convex side of the seed, levelled with the outer surface at one extreme and forming a crest near the germinative plug (figure 1b, c). The light colour of the raphe is due to the lack of inner seed coat tissue below it (figure 1e). The embryo is conferruminate, being comprised by a single hard, undifferentiated cream coloured body where the cotyledons and the embryonic axis are fused (figure 1d, e).

Morphologically, germination begins through radicle protrusion and the formation of the germinative button that closely follows (figure 1f). After an initial period of axial root growth (figure 1g, h) a scarlet epicotyl, comprised of a crown of cataphyls that surround the apical meristem, emerges 1 (figure 1i, j). Epicotyl growth is slow and frequently suppressed, with many cases of necrosis and subsequent resprouting. Some individuals presented resprouting from the upper part of the taproot and originated normal seedlings. The development of the radicular system, in its turn, is intense, with one or more slender, lignified and abundantly ramified taproots. After about one year, the epicotyl resumes its growth (figure 1k); first leaves spread open after a gradual development of a series of
DESICCATION AND STORAGE OF *EUGENIA DYSENTERICA* SEEDS

Figure 1. Morphology of seed and post-seminal development of *Eugenia dysenterica*. Seed: (a) and (b) external views; (c) detail of raphe near the germinative plug; (d) longitudinal section; (e) transversal section showing the lack of inner seed coat tissue below raphe. Germinating seed: (f) radicle protrusion; (g) and (h) axial root growth; (i) and (j) emerging epicotyl; (k) maximum epicotyl growth before leaf emergence; Seedling: (l) general appearance. Note necrosed epicotyl. Scale bars: 5 mm.
cataphyls. Seedlings (figure 1l) are cryptogeal (sensu Duke and Polhill, 1981), with long, slender and dark-brown axial roots, with many whitish-yellow hairy secondary roots. The epicotyl is erect, cylindrical and slender, brownish near its base and light green near the apex, downy with tiny white hairs. A sequence of distichous cataphyls gives place to the eophylls, which are glabrous, lanceolate, with the apex and base acute and integument margins and are green, distichous on trunk.

Germination at constant temperature regimes
Germination percentage was highest at temperatures between 15°C and 25°C (P≤0.001) (figure 2). At this optimum interval almost all seeds germinated within 40 days. Germination was reduced at higher temperatures (35°C) and the temperature of 40°C was lethal (browning seed covered by fungi). Temperatures lower than 20°C retarded germination by increasing the time lag to onset of germination, reducing the germination rate and lowering the final germination percentage (except at 15°C). From the linear regressions of the relationship between constant temperatures and germination rate (figure 2), it was possible to estimate the optimum temperature, which corresponds to the intersection of the two regression lines (24.1°C).

The optimum range of temperatures for seed germination of *E. dysenterica* agrees with a number of other cerrado woody plants, *i.e.*, it lies between 20°C and 35°C (Joly, Felippe, Dietrich and Campos-Takaki, 1980; Arasaki and Felipe, 1987). At Low temperatures (10-15°C) seeds of *E. dysenterica* are able to germinate perfectly, but the germination rate is reduced and the time lag to onset the germination is increased, which
reflects a slowing down of biological processes. Low temperatures might inhibit activities of virtually all (or many) enzymes thus slowing protein synthesis and other biosynthetic processes necessary for germination and seedling development (Kamaha and Maguire, 1992). The highest temperature (40°C) had a deleterious influence on seed germination perhaps because most enzymes are inactive at such temperature. The primary site of high temperature sensitivity in germinating seeds is thought to be closely concerned with a lower rate of protein synthesis by the embryo at this temperatures (Riley, 1981).

**Effects of desiccation**

Of the two methods of desiccation used, the drying rate at 24°C and 15% RH was the faster, especially after 10 days of drying, falling from 44-46% moisture content to less than 20% in 28 days (figure 3). At 15°C and 20% RH, moisture content of seeds was reduced to 22% after 39 days.

*E. dysenterica* seeds present relatively high moisture contents (47-53%) on shedding. The response of seed germination to drying to various moisture contents was sigmoidal (figure 4a), typical for recalcitrant seeds, with no survival to moisture contents as low as 18-22% (lethal moisture content). Germination was reduced when moisture content fell from 47% to 43%, which corresponded to the upper inflection point on the sigmoidal curve (figure 4a) (critical moisture content). Percentage germination reached 50% when seeds had moisture contents near 36-38%. Within the limits of the present experiments, a significant relationship between moisture content reduction and viability loss (germination) on a probability scale was linear (P≤0.05) and independent of the drying rate. The moisture content at which seed viability was completely lost in this species is similar to other tropical species with recalcitrant seeds, such as 18-20% for *Hevea brasiliensis* (Chin, Aziz, Ang and Hamzah, 1981), 15-20% for *Hopea odorata* (Corbineau and Côme, 1988) and 21% for another tropical-Myrtaceae species, *Myrciaria cauliflora* (Valio and Ferreira, 1992).

![Figure 3. Drying curves of *E. dysenterica* seeds dried at 24°C and 15% RH (O) and 15°C and 30% RH (●).](image-url)
Figure 4. The relationship between moisture content and seed germination (linear percentage scale, a; probability scale, b) of *E. dysenterica* seeds dried at 24°C and 15% RH (O) and 15°C and 30% RH (●). Extreme values on the probability scale are indicated by arrows.

Figure 5. The relationship between embryo moisture content and water potential equilibrated in PEG solutions at 10°C. Line fitted by eye.
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From the moisture sorption isotherm determined in figure 5 the estimated critical moisture content corresponds to a range of water potentials from -0.8 to -1.9 MPa (relative humidity values near to 99.4-98.7%). Therefore, this range of values is lower than the critical water potentials observed in temperate recalcitrant seeds of Quercus rubra (Pritchard, 1991) and Araucaria hunsteinii (Pritchard, Tompsett, Manger and Smidt, 1995) but similar to the tropical recalcitrant seeds of Theobroma cacao and comparable to the permanent wilting point of many growing tissues (Roberts and Ellis, 1989).

Individual seed mass and moisture content vary greatly (figure 6). According to Probert and Longley (1989) both might be considered as key sources of apparent variability in desiccation tolerance between seeds (figure 4b). Similar findings have been reported for other recalcitrant seeds (Tompsett, 1986; Finch-Savage, 1992). The relatively large coefficient of variation of moisture content (22.35%) may be due to the variation of seed mass (figure 6) and the heterogeneity of seed development time in trees, two common features of the recalcitrant seeds of wood perennials (Chin, 1988; Tompsett and Pritchard, 1993).

Species that produce recalcitrant seeds are typically originated from humid tropical areas where the environment suitable for seedling growth is more or less continuous throughout the year (Roberts and King, 1980). Although presenting recalcitrant seed storage behaviour, E. dysenterica presents traits that enable it to cope with the harsh climate of the cerrado regions; flowering occurs from August to October and the mature fruits are shed from October to December, during the rainy season (Lorenzi, 1992). This phenological schedule corresponds to a strategy thought to maximise the survival of seeds after dispersion by birds and small mammals (Gottsberger and Silberbauer-Gottsberger, 1983) in the dry habitat of the cerrado, which is rainless for at least 6-8 months per year (Ratter et al., 1997).

Figure 6. The relationship between individual mass and moisture content for E. dysenterica seeds. Coefficient of variation of individual seed moisture content (CV_{m.c.}) and seed mass (CV_{mass}) inside the box.
**Effects of storage temperature**

Although some seeds of recalcitrant species of tropical origin tend to suffer chilling damage when stored at temperatures of about 15°C and below (Chin and Roberts, 1980), our results showed that fully hydrated *E. dysenterica* seeds were chilling-tolerant since there was no significant difference between storage temperatures (P>0.05). A significant relationship between viability loss (germination) and time of storage on a probability scale was linear (P≤0.05); a significant reduction of percentage germination was first detected at 100 days of storage (figure 7). Viability was reduced to 50% after approx. 175 days of storage and all seeds failed to germinate after 300 days of storage. At the highest storage temperature, seeds initiate germination inside the polythene bags (50 days, 4%; 100 days, 21%; 150 days, 17%; 200 days, 33%; 250 days, 27%). The speed of spontaneous germination during storage depends on the moisture content of the seed and on the storage temperature used (Pritchard *et al*., 1995). Given that *E. dysenterica* seeds germinated at temperatures near to 10°C, it is surprising that spontaneous germination in storage at 10°C did not occur during 350 days (figure 7). Spontaneous germination might be prevented by storage at temperatures lower than 10°C.

![Figure 7](image_url)

**Figure 7.** The effect of storage on germination (probability scale; a, b, c) and moisture content (d, e, f) of *E. dysenterica* seeds. The seeds were stored at temperatures of 5°C (a, d), 10°C (b, e) or 15°C (c, f). Extreme values on the probability scale are indicated by arrows. Vertical bars represent one s.e. of the mean (where these are larger than symbols). Dotted line represents initial moisture content (45%).
There is no satisfactory method for long-term storage in recalcitrant seeds since they may not be dried to low moisture contents or be stored at sub-zero temperatures. Moist storage at temperature ranging from 3°C to 20°C, has been achieved for very brief periods in some tropical species such as *Symphonia globulifera* (Corbineau and Côme, 1986) and *Hancornia speciosa* (Oliveira and Valio, 1992). Chin and Roberts (1980) have recommended the storing of recalcitrant seeds in a wet medium to avoid desiccation but this method has not been successful for many species because of various problems such as fungal contamination and germination during storage. Pritchard *et al.* (1995) showed approx. 15% radicle pre- emergence when hydrated *Araucaria hunsteinii* seeds were stored at 21°C after 6.6 months. Similar to Hor, Chin and Zain Karin (1984) and Andrade (2001), it was found that moisture content variation among the three different storage temperatures rarely exceeded 1.7% (figure 7d, e, f). We believe that the decline in germination percentage during storage was not due to microbial attack since fungal contamination in *E. dysenterica* was only observed at 15°C, after 250 days of storage, although seeds were treated with Benomyl.

In conclusion, the results demonstrate that *E. dysenterica* seeds are desiccation sensitive and not able to maintain high viability for more than 150 days when stored in a hydrated state (45% moisture content), independent of storage temperature. The assessment of seed viability may be carried out at temperatures ranging from 15°C to 25°C, but preferentially at 24.1°C (thermal optimum for seed germination). One possible future approach to increase longevity and control seed germination during storage would be the use of the partial desiccation method before storage (Chin, 1988), since in some cases [(*Theobroma cacao*; Hor *et al.*, 1984), (*Hevea brasiliensis*; Chin, 1988), (*Euterpe edulis*; Andrade, 2001)], this method has been successful in prolonging the life of stored seeds when compared with the imbibed storage.

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