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Effect of seed desiccation and sucrose concentration on the *in vitro* establishment of mangabeira (*Hancornia speciosa* Gomes var. *gardneri*) seedlings

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Mangabeira (*Hancornia speciosa* Gomes var. *gardneri*) is one of the most important fruit trees in the cerrado biome, its fruits being highly valued both for *in natura* consumption and for processing. Although its seeds are delicate, losing viability within a few hours after collection, seeds still represent the predominant tool of propagation multiplication of this species, since the plant encounters limitations in the formation of adventitious roots, hindering vegetative propagation. Thus, the present work aims to evaluate the *in vitro* establishment of Mangabeira seedlings as a function of the extent of natural desiccation and the sucrose concentration in the culture medium. Four sucrose concentrations (15, 30, 45 and 60 g L^{-1}) and four natural drying periods (24, 48, 96 and 192 h after the seed pulping) were used in a factorial experiment. 60 days after in vitro culturing of the naked embryos, the following variables were evaluated: germination %, germination speed index (GSI), mean germination time (MGT), oxidation index, culture medium contamination index, length of the largest root, stem diameter, seedling height and number of live and dead leaves. The interaction between desiccation times and sucrose concentrations was not significant for any of the variables analyzed. Germination was influenced only by the desiccation time, being reduced after 106 hours of natural drying. Increasing concentrations of sucrose up to 60 g L^{-1} reduced GSI and seedling height. In contrast, leaf mortality decreased, which contributed to the production of seedlings with greater ability of acclimatization to field conditions.

Key words: Germination, *in vitro* cultivation, desiccation, *Hancornia speciosa* Gomes.

INTRODUCTION

Mangabeira (*Hancornia speciosa* Gomes var. *gardneri*) is a tropical fruit tree of medium size, spontaneously vegetating in several regions of Brazil (Lorenzi, 2002; Costa et al., 2011). The fruits vary from round to oval, with a diameter of 2-6 cm, and white, soft fibrous pulp, enclosing 2-15 flattened, discoid seeds (Lorenzi, 2002; Santos et al., 2010). Fruit harvesting usually begins in November-December and extends to May-June (Vieira

Neto, 2002).

All parts of the mangabeira tree produce white or pale pinkish latex that can be exploited in the production of rubber, in folk medicine for the treatment of warts, in waterproofing of fabrics and in the production of bags (Barros et al., 2010; Silva et al., 2011). The tree can also be used as firewood and in the reforestation of deteriorated areas. However, fruits are the main product, being extremely appreciated for their aroma and nutritional value. Fruits have many uses; they can be consumed fresh or rather used in, the manufacture of soft drinks, ice creams, jams, syrups and the preparation of wine (Carnelossi et al., 2009; Barros et al., 2010).

The propagation of this species by traditional methods has been hampered by the fact that its seeds are delicate, rapidly losing their viability as soon as they are removed from the fruit (Santos et al., 2010). Another factor that hinders propagation is the inhibitory action of the fruit pulp on seed germination. Hence, there is a need for a thorough investigation to evaluate the sensitivity to desiccation of the seeds of this species (Bovi et al., 2004; Barrozo et al., 2014). Nevertheless, in view of the great diversity of the native tropical tree species in Brazil, whose seeds are intolerant to desiccation, it is necessary to develop specific technologies for conservation during storage (Maluf et al., 2003), or maintaining the viability of the seeds that are subjected immediate germination rapidly after fruit collection.

An efficient way to propagate the delicate species, such as mangabeira, is the *in vitro* establishment of seeds. This technique allows maximizing the germination rate, production of uniform seedlings with adequate genetic and phytosanitary quality (Stein et al., 2007). Moreover, *in vitro* cultured seeds exhibit greater germinability than those raised in nurseries, because the *in vitro* conditions are more suitable for germination processes and early seedling development (Noleto and Silveira, 2004). The application of tissue culture techniques to cerrado fruit trees can promote systematized multiplication of plants, exchange of genetic material, germplasm rescue and preservation of a threatened material. In these species, the *in vitro* germination has performed important achievements, allowing, in addition to other landmarks, the overcoming of dormancy within short time (Pinhal et al., 2011).

In vitro cultivation requires an exogenous source of energy to the plants, since in these environments plants have limited photosynthetic activity. Sucrose has been the most widely used carbon source, added to culture media in concentrations ranging from 20 to 40 g L^{-1} (Ferreira et al., 2002); which might affect the osmotic potential of the medium and hence the metabolism and growth of the cultured plant (Reis et al., 2008). Therefore, the objective of this study is to evaluate the impacts of both natural desiccation of seeds and the sucrose concentration in the culture medium on the *in vitro* establishment of Mangabeira (*H. speciosa* Gomes var. *gardneri* (A.DC).

MATERIALS AND METHODS

Fruits of Mangabeira (*H. speciosa* Gomes var. *gardneri*) were obtained in November 2015 from the germplasm collection of the Agronomy School of the Federal University of Goiás (EA/UFG, Brazil) established in December 2005. After collection, the fruits were sent to the EA/UFG biotechnology laboratory, where they were washed and stored in plastic containers for 6 days, at 8ºC. The fruits were then manually pulped and washed in running tap water until the pulp was completely removed and placed on paper towel for natural drying for 24, 48, 96 and 192 h at 25°C and 65% relative humidity. Seeds with no injuries or mechanical damage were selected. Twenty seeds from each drying period were randomly selected for moisture content determination in an oven at 105°C for 24 h.

Seed coat was removed by hand, and desiccated seeds were disinfected following the aseptic methodology of Vieira (2014). After seed coat removal, the naked embryos were washed in distilled water, and then in autoclaved water containing commercial detergent. Subsequently, the seeds were rinsed three times with distilled water, followed by immersion in 70% alcohol for two minutes and in 100% sodium hypochlorite for five minutes and finally by three times rinsing in distilled water to remove excess hypochlorite.

In a laminar flow chamber, the embryos were immersed for 30 min in 100% sodium hypochlorite, followed by three rinses with distilled and autoclaved water to remove excess hypochlorite. The embryos were then incubated in 200 mL glass vials containing, 30 mL of half-strength macronutrient solution of Murashige and Skoog culture medium (Murashige and Skoog, 1962), amended with 0.10 mg L^{-1} pyridoxine; 1.0 mg L^{-1} thiamine; 0.10 mg L^{-1} myo-inositol, 2 mg L^{-1} indolebutyric acid (IBA), 1 g L^{-1} activated charcoal, and different concentrations (15, 30, 45 and 60 g L^{-1}) of sucrose.

The experiment was factorial, with two factors and 10 replications in a completely randomized design. The main factors were desiccation period with four levels (24, 48, 96 and 192 h) and sucrose concentration with four levels (15, 30, 45 and 60 g L^{-1}). Each replicate was a 200 mL glass vial containing 30 mL of the modified Murashige and Skoog culture medium and inoculated with one Mangabeira embryo.

The inoculated glass vials were incubated in the dark for 48 h at a temperature of 25 ± 2°C, and then under a light period of 16 hours and photon flux density of 35 µmol m^{-2} s^{-1} for 60 days. The cultures were investigated daily, to determine the oxidation index (OI), contamination index (CI) and to identify the contaminant fungi and/or bacteria. At the end of incubation period, germination speed index (GSI), mean germination time (MGT), seedling height, stem diameter, number of live and dead leaves per seedling and length of the largest root.

Germination speed index was calculated using the following equation: $\mathrm{GSI} = \sum_{i=1}^{n} (\frac{G}{N})$, described by Maguire (1962), where G

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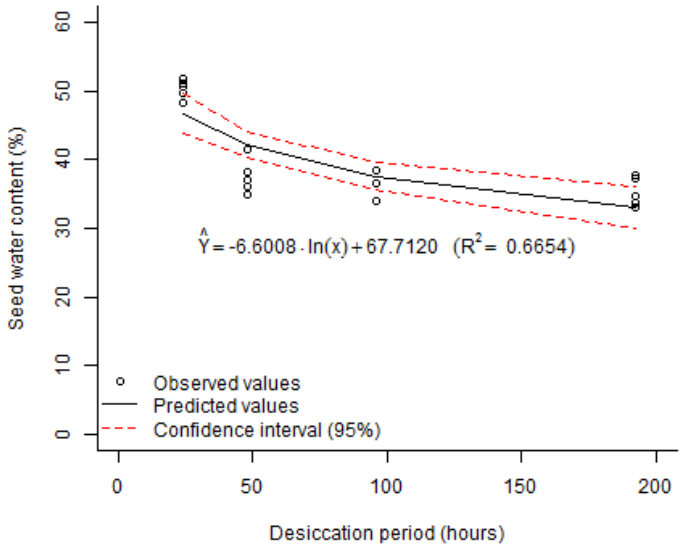

Figure 1. Water loss in mangabeira seeds (*Harconia speciosa* var. g*ardneri*) submitted to different periods of natural desiccation.

represents the number of normal seedlings germinated per day, divided by the number of days N elapsed between sowing and germination. The mean germination time was obtained from the equation: $\mathrm{MGT} = \frac{\sum_{i=1}^{n} (G\ x\ N)}{T}$, where G represents the number of normal seedlings germinated per day, N is the number of days elapsed between sowing and germination and T is the total number of germinated seeds (Labouriau, 1983). The length of the largest root (cm) was obtained by measuring the primary roots with graduated ruler. Also, the percentage of contamination and oxidation of the medium during the experimental period was determined.

Data were submitted to analysis of variance using the statistical program R Core Team version 3.3 (2016). When there were significant differences between treatments, regression analysis was used for determination of the optimum sucrose concentration and inoculation time after pulping for the *in vitro* establishment of mangabeira seedlings.

RESULTS AND DISCUSSION

There was non-significant interaction ($p > 0.05$) between the seed desiccation period and sucrose concentration of the medium on the evaluated variables. Regression analysis revealed a pronounced loss of seed water from 50.22 to 35% with the increase in drying time from 24 to 192 h (Figure 1).

The water loss by seeds was crucial for seed germination and seedling development (in terms of the number of viable leaves) (Figure 2). A trend of quadratic behavior was observed between the extent of desiccation in one hand and seed germination and seedling development in the other hand; that is mangabeira seeds attained maximum germination potential at 106 - 110 h of natural desiccation (equivalent to 36.8% seed water content). Regarding the seedlings originating from seeds dried for periods longer than 110 h, the marked reduction in the number of viable leaves means reduced seedling vigor caused by the decrease in the water content of seeds with the progress of desiccation period.

Barros et al. (2010) found that desiccation of mangabeira seeds for periods longer than 36 h (seed water content < 25.7) reduced seedling emergence. Poor seedling vigor and difficulty in maintaining leaves viable during the *in vitro* establishment were also observed in guariroba seeds kept at a constant temperature of 37ºC for a period of 12 days after harvest (Rubio Neto et al., 2015). Salomão et al. (2004), studying the effect of desiccation of mangabeira seeds on their viability, verified that water content lower than 26% fresh weight impaired germination capacity, and values below 11% led to complete loss of viability.

The mean germination time as well as the oxidation and contamination indexes were non-significantly affected by treatments and showed average values of 19.32, 1.85 and 2.50%, respectively, regardless of desiccation period and sucrose concentration of the medium. The removal of seed coat allows high efficiency of the disinfection protocol; thus lowering the opportunity of microbial growth and guaranteeing insignificant indices of contamination of the embryo culture. Although seed coat can serve as a physical barrier for water entry and also as a shelter against attack of pathogens, yet the presence of seed coat in embryo cultures might participate in infection of the culture with recalcitrant pathogens. The maintenance of the seed coat in seeds of *Mouriri elliptica* grown *in vitro* significantly increased the rate of culture contamination, reducing the viability of the embryo (Lima et al., 2016). Although mature mangabeira seeds possess high water content, which can promote

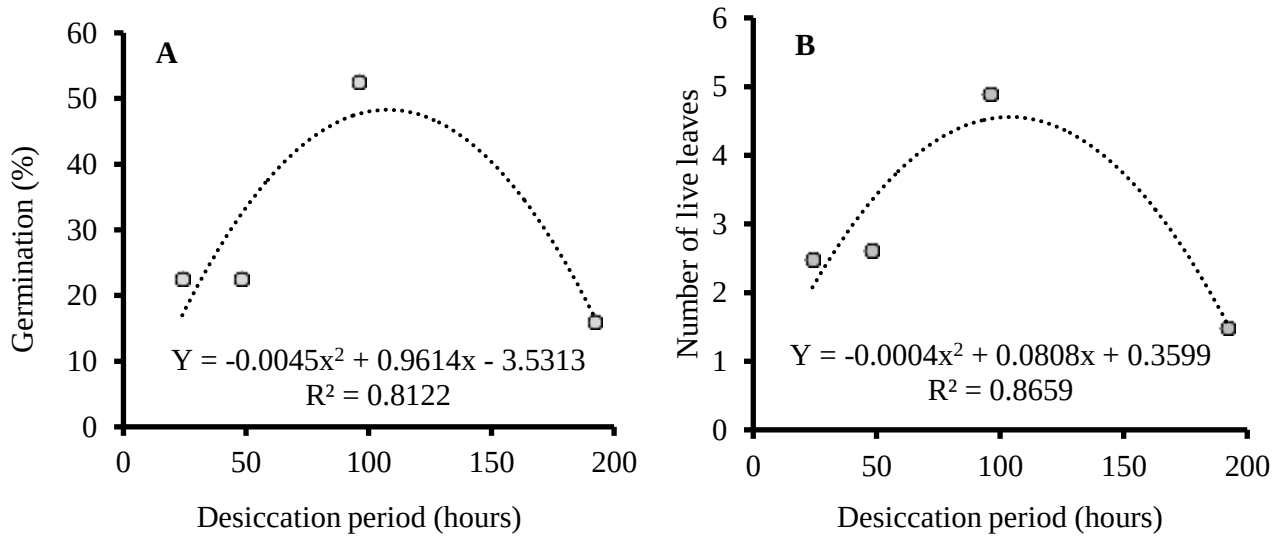

Figure 2. Germination percentage (A) and number of live leaves (B) of mangabeira seedlings grown *in vitro* up to sixty days after inoculation.

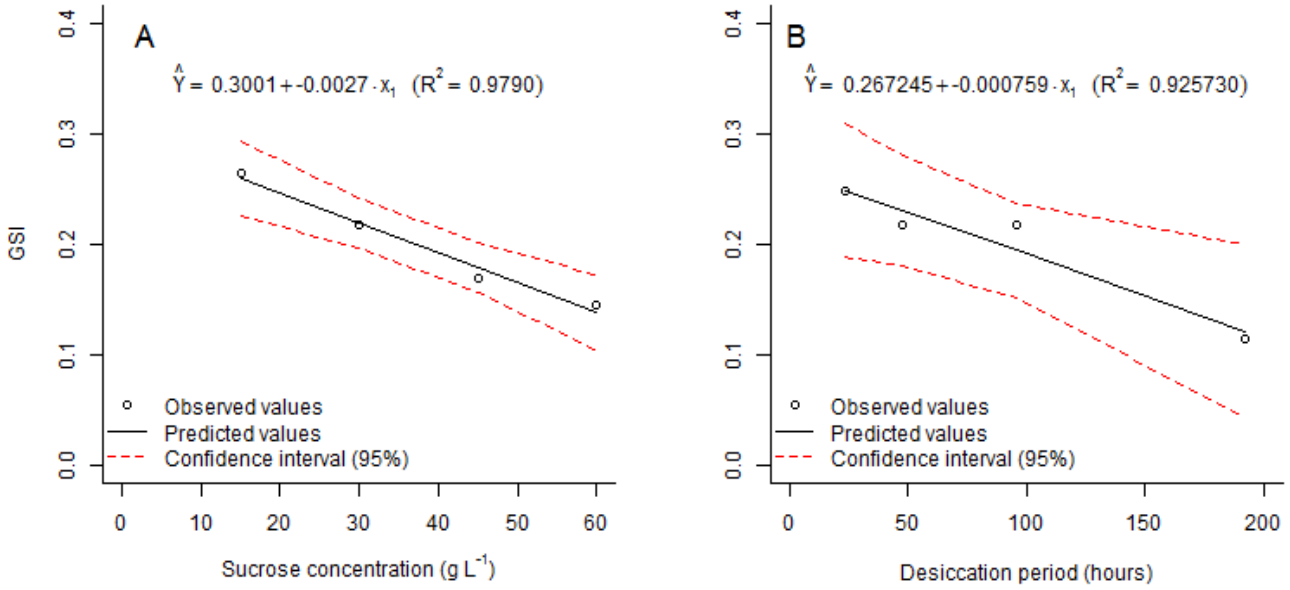

Figure 3. Influence of the increasing sucrose concentrations in the medium (A) and the seed inoculation period after pulping (B) on the germination speed index of mangabeira seedlings grown *in vitro*.

high rates of culture contamination (Rubio Neto et al., 2015), no influence of this factor was verified on the medium contamination, since, regardless of the desiccation period, the contamination index remained below 2%.

The germination speed index (GSI) decreased with increasing either the sucrose concentration or the time between fruit pulping and seed inoculation (desiccation period) (Figure 3). The addition of solutes to the culture medium, such as macronutrients, sucrose and others, leads to a considerable decrease in the osmotic potential of the medium due to the lowering of water potential (Kerbauy, 2012), however, in this study, the concentrations of these elements were not sufficient to impose water stress capable of counteract the strong forces of imbibition prevalent during germination.

Contrary to what was observed in the present study, pequi seeds (*Cariocar brasiliense* CAMB.) exhibited

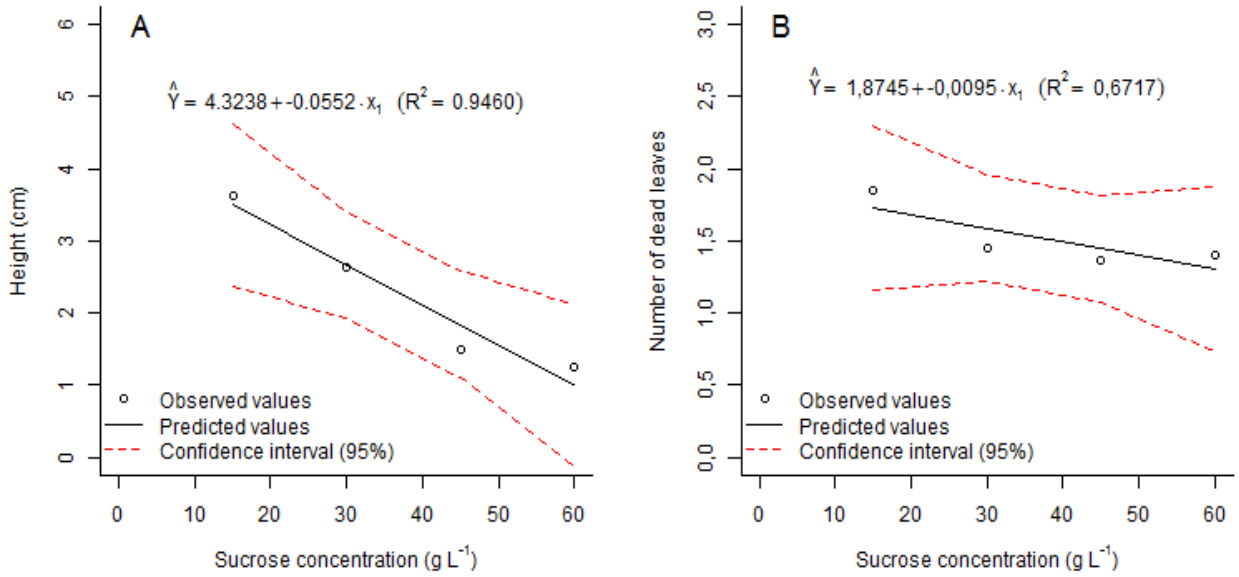

Figure 4. Height of mangabeira seedlings (A) and number of dead leaves sixty days after inoculation.

increases both in the germination percentage and the germination speed index with increase in desiccation time (reduction of seed water content) (Silva et al., 2013). These contrasting differences among native Cerrado species in response of seed germination to desiccation are mainly due to the anatomical differences among fruits and seeds and the residual water content remaining in the seeds after harvest.

Increasing sucrose concentration of the medium influenced also seedling height and the number of dead leaves. The height of the mangabeira seedlings seems a secondary response, being dependent on the presence of live leaves. This suggests that in seedlings with fewer dead leaves, sucrose consumption for maintenance of live leaves increased, which deprives the meristem from sucrose and this can partially explain the reduction in plant height (Figure 4). The reduction in the number of dead leaves with the increase in sucrose concentration of the medium is interesting, because it allows obtaining seedlings with greater photosynthetic area, which may favor their acclimatization.

Mangabeira seeds, thus, exhibit short postharvest longevity, which necessitates immediate sowing after extraction from the fruit. The seeds are also considered delicate, suffering intrinsic damage leading to loss of viability and vigor with reduction of seeds moisture. The gradual decrease of GSI and germination percentage and the increase of the number of dead leaves support this conclusion, showing that even under controlled conditions, such as the *in vitro* cultivation, the resumption of seedling development is dependent on the conditions of storage and conservation of the embryo. Seed drying, although not reducing the seed water content below 26%, was considered critical by Salomão et al. (2004), possibly causing damage to vital seed tissues, such as the embryo, which would explain the drastic reduction of seedling germination after 106 h of drying (Figure 2A).

In potato plants cultured *in vitro*, Bandinelli et al. (2013) observed that the increase of sucrose concentration in the MS medium was accompanied by a decrease in the biomass production. The dynamics of solute translocation in the phloem helps to explain the reduction in the height of seedlings cultured in medium with higher concentrations of sucrose. In the age at which the mangabeira seedlings were cultured, the photosynthetic rate could be considered null, since the leaves were not yet fully developed. Thus, the phloem loading with sucrose and other solutes from the source (culture medium) occurred in the apical direction; these solutes being intercepted by the proximal sink organs. It was observed that with the increase in sucrose concentration of the medium, there was a reduction in the number of dead leaves per seedling, concomitant with an increase in the number of young leaves which act as a sink, resulting in smaller amounts of organic solutes reaching the apical meristem. Lower seedling height can be interpreted as a consequence of maintenance of live leaves.

The length of the largest root and the stem diameter did not differ between the sucrose concentrations and the inoculation periods after pulping. This is due to the fact that the increase in sucrose concentration of the medium was accompanied by reduction in the mortality of young leaves (sink). These dynamics possibly attenuate the competition between the different sinks (young leaves and meristems), favoring growth of young leaves at the expense of limiting the amount of organic solutes reaching the apical root meristems. However, the

secondary growth (stem diameter) of seedlings was less influenced by the medium conditions at the beginning of the establishment period; it may be manifested at the later developmental stages of the plant, or after the acclimatization period.

Conclusions

The natural drying of seeds of *H. speciosa* Gomes var. *gardneri* can be carried out up to 106 h after their extraction from the fruit (36.8 water content) without prejudice to the *in vitro* germination rate and the maintenance of live leaves of the seedlings. Increasing sucrose concentration up to 60 g L^{-1} reduced germination speed and seedling height. Leaf mortality was also reduced, contributing to the production of seedlings that are more capable of acclimatization to field conditions.

Conflict of Interests

The authors have not declared any conflict of interests.

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