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CRYOPRESERVATION

# *In vitro* germination of zygotic embryos excised from cryopreserved endocarps of queen palm (*Syagrus romanzoffiana* (Cham.) Glassman)

**Izulmé Rita Imaculada Santos**[1] · **Antonieta Nassif Salomão**[1]

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**Abstract** Queen palm (*Syagrus romanzoffiana* [Cham.] Glassman) is a palm species best known as an ornamental tree for urban landscaping, but recently, it has been evaluated as a potential crop for biofuel production. The objective of the present work was to establish a cryopreservation technique for queen palm to ensure long-term conservation of this species. The cryopreservation protocol consisted of direct immersion in liquid nitrogen (LN) of whole endocarps with water contents ranging from 5.5 to 10.9%, followed by slow thawing at room temperature (25 ± 2°C) excision and *in vitro* culture of zygotic embryos. Viability of zygotic embryos isolated from endocarps with different water contents was evaluated before (control) and after freezing in LN using *in vitro* culture on Woody Plant Medium (WPM) medium. Germination percentages of zygotic embryos isolated from endocarps stored in LN varied from 84 to 93%, whereas those isolated from controls ranged from 55 to 71%. Germination rates were significantly higher for zygotic embryos excised from cryopreserved endocarps. The water content of control or frozen endocarps did not have a significant effect on germination percentages of zygotic embryos. Zygotic embryos excised from endocarps following cryopreservation in liquid nitrogen developed into normal plantlets after *in vitro* culture. The technique tested is simple, efficient, and can be used in plant gene banks as a routine approach for long-term conservation of queen palm germplasm.

**Keywords** Cryopreservation · Zygotic embryos · Queen palm · *Syagrus romanzoffiana*

✉ Izulmé Rita Imaculada Santos
izulme.santos@embrapa.br

[1] Embrapa Recursos Genéticos e Biotecnologia (Embrapa Genetic Resources and Biotechnology), Parque Estação Biológica (PqEB), Avenida W5 Norte, final, S/N, Asa Norte, CEP, Brasília, DF 70770-917, Brazil

## Introduction

Queen palm (*Syagrus romanzoffiana* [Cham.] Glassman) is a perennial palm tree of the Arecaceae family widely distributed in South America in tropical and subtropical areas (Glassman 1987; Begnini *et al.* 2013). This species has many uses. It is a popular ornamental tree in urban landscaping due to its beautiful appearance and low maintenance requirements. The tree is also a source of edible palm hearts (apical meristem and undifferentiated leaves) that are consumed as a gourmet vegetable, of fruits that are sought by birds and small mammals, and of leaves that are used as feed for cattle (Galetti *et al.* 1992; Lorenzi *et al.* 1996; Lorenzi 2002). More recently, there has been increasing interest in using this species for biofuel production due to the high lipid content of the endosperm, which can exceed 50% (Moreira *et al.* 2013). Another important trait of the queen palm is that it can grow in many different types of environments, even dry areas, producing abundant fruits throughout the year.

The queen palm produces fleshy fruits, that become bright orange, juicy, and sweet when ripe and that consist of a thin layer of fibrous flesh (mesocarp), a woody endocarp, and a single seed generally with one embryo (Goudel *et al.* 2013). The main propagation method of the species is sexual through seed germination. However, seedling production is limited by uneven seed germination, slow seedling emergence, and high seed loss due to deterioration (Goudel *et al.* 2013). Studies on longevity and germination patterns of stored queen palm seeds indicate that they are short lived, with uncertain storage behavior. Seed germination percentages decrease to very low levels after storage under laboratory conditions, most likely

due to loss of embryo vigor and deterioration caused by crystallization of lipids of the seed endosperm (Balesevic-Tubic *et al.* 2010; Goudel *et al.* 2013; Oliveira *et al.* 2015). Therefore, preservation and conservation of *S. romanzoffiana* via orthodox seed storage methods is not feasible (Sturião *et al.* 2012; Goudel *et al.* 2013; Oliveira *et al.* 2015).

There is increasing interest in the development of effective protocols for germplasm preservation of palm species in general and of *S. romanzoffiana* in particular (Kaviani 2011). However, presently *ex situ* conservation of queen palm accessions can only be implemented through establishment of germplasm collections in field gene banks, which exposes plants to many threats and is not a long-term conservation strategy (Kaviani 2011). In cases like this, cryopreservation in liquid nitrogen (LN) has been appointed as the most promising alternative strategy for long-term storage of valuable plant germplasm. Cryopreservation involves storage of plant material at ultra-low temperatures in liquid nitrogen (−196°C), and at this temperature, cell division and metabolic activities are suspended and the samples can be stored without changes for long periods of time. It requires limited space, protects samples from contamination, requires very little maintenance, and is a cost-effective option for plant genetic resources conservation.

Embryo culture *in vitro* has been used in seed conservation as an alternative, rapid method of assessing seed quality or to recover growth of difficult-to-store seeds, and generally, there is a good correlation between embryo growth *in vitro* and germination percentages (Pritchard *et al.* 2000). Cryopreservation of seeds and zygotic embryos was previously used as an alternative strategy for long-term conservation of many plant species (Pritchard *et al.* 2000; González-Benito *et al.* 2009; Santos and Salomão 2016). In the case of palms, cryopreservation protocols have been developed for seeds and embryos of different species (Grout *et al.* 1983; Chin *et al.* 1988; Assy-Bah and Engelmann 1992; Engelmann *et al.* 1995; Raja *et al.* 2003; Steinmacher *et al.* 2007; N'Nan *et al.* 2012). However, there is no report of cryopreservation of queen palm germplasm.

The objective of this study was to establish a cryopreservation technique for queen palm endocarps to ensure long-term conservation of this species. This paper describes the effect of water content and storage in LN on both *in vitro* germination of queen palm zygotic embryos and seedling development. This is the first report on cryopreservation of *S. romanzoffiana* germplasm.

## Materials and Methods

**Plant material** Mature fruits of queen palm [*S. romanzoffiana* (Cham.) Glassman] were collected from trees growing in Brasília, Federal District, Brazil, and taken to the laboratory at Embrapa Genetic Resources and Biotechnology, in the same locality. Fruits were processed immediately to remove the juicy pulp (mesocarp) surrounding the endocarps first using a blender and subsequently by rubbing fruits across a metal mesh sieve. Endocarps were washed with a sodium hypochlorite solution [2 to 2.5% active chlorine, Qboa®, Indústrias Anhembi Ltda, Osasco, SP, Brazil) containing five to eight drops (300 to 500 μL) of commercial grade detergent (Ypê, Química Amparo Ltda, São Paulo, SP, Brazil) and then rinsed thoroughly under running tap water until all visible traces of the fibers had been removed. Endocarps were spread out on the laboratory bench covered with sheets of germination paper, at room temperature (25 ± 2°C), where they were maintained for 24 h to dry. Three replicates containing 15 de-pulped (mesocarp removed) endocarps were used in all the tests carried out in this study.

**Water content determination and adjustment** The initial water content (WC) of freshly harvested (FH), de-pulped, endocarps was determined by the oven method (Brazilian Ministry of Agriculture Livestock and Supply 2009) using a sample of 15 endocarps (3 × 5) and calculated on a fresh weight basis (FWB). Afterwards, the WC of endocarps was adjusted to four moisture levels using dehydration or hydration. For dehydration, endocarps were placed inside a plastic container and covered with 200 g of blue silica gel (commercial grade, Vetec®, ref. 155, Rio de Janeiro, RJ, Brazil) before the container was closed with its lid and kept in a germinator for 24 h, in darkness, at a constant temperature of 25 ± 2°C. For hydration, endocarps were placed over the nets of an acrylic germination box (Gerbox®, São Paulo, SP, Brazil) containing 200 mL of distilled water before the box was covered with its lid and kept in a germinator (Eletrolab®, São Paulo, SP, Brazil) for 24 h, in darkness, at constant temperature (25 ± 2°C) for 21, 24, or 48 h. Water content of dehydrated and hydrated endocarps was determined by the oven method as described previously.

**Freezing and thawing** For freezing, endocarps of different WC were transferred to trifoliate envelopes (Moore and Buckle, Ltd., St. Helens, UK), heat sealed, transferred to canisters, and submerged in LN for 6 mo. Thawing was done by removing the samples from the cryotank and maintaining them at 25 ± 2°C for at least 4 h.

**Zygotic embryo excision and *in vitro* culture** Viability of zygotic embryos (ZEs) excised from endocarps with different WC was assessed before and after freezing in LN by means of *in vitro* germination. Information on *in vitro* culture of *S. romanzoffiana* ZE is scarce, so a preliminary test was carried out to test two culture media: Murashige and Skoog (1962) (MS) and Woody Plant Medium (WPM) (Lloyd and

McCown 1981). ZE cultivated on WPM (data not shown) medium showed the best germination results; therefore, this medium was used in all the remaining experiments. For each WC level, 45 (3 × 15) ZEs were excised from control endocarps and 45 (3 × 15) ZEs were excised from endocarps stored in LN. Before excision of ZE, endocarps (controls and frozen) were decontaminated to avoid contamination during *in vitro* culture with a sodium hypochlorite solution (2.0 to 2.5% active chlorine, Indústrias Anhembi Ltda) containing 8–10 drops (500 to 625 μL) of commercial grade detergent and 5–8 drops (300 to 500 μL) of Tween® 20 (P7949, Sigma-Aldrich®, St. Louis, MO) for 15 min, under constant agitation in a rotary shaker. In the laminar flow hood, endocarps were rinsed three times with sterile, distilled water. All endocarps were hydrated by adding 100 mL of sterilized distilled water to the endocarps before they were transferred to a seed germinator, in darkness, at a constant temperature of 25 ± 2°C for 48 h. After hydration treatment, endocarps were cracked using a sterilized bench-top vise in the laminar flow hood to extract the seeds. A whole ZE (3 to 5 mm long) was excised from each seed. After excision, individual ZEs were placed into 25 × 150 mm glass test tubes (Uniglass, Piracicaba, SP, Brazil) containing 10 mL of WPM. The WPM contained 30 g $L^{-1}$ sucrose and 3 g $L^{-1}$ activated charcoal, added to prevent browning of ZE during culture, and solidified with 7.0 g $L^{-1}$ plant cell culture-tested agar (A1296, Sigma-Aldrich®). All chemicals used in the medium composition were made by Sigma-Aldrich® Chemicals Co. and purchased in Brazil, from a local representative. The pH of the medium was adjusted (by adding 1.0 N NaOH solution) to 5.8 before the addition of agar and before sterilization by autoclaving at 121°C and 100 kPa of pressure for 20 min. All cultures were maintained in a growth room at 25 ± 2°C and 12-h photoperiod of 50 μmol $m^{-2}$ $s^{-1}$ light intensity provided by cool white fluorescent tubes (Sylvania, daylight plus FHO85 WT12®, Sylvania Brasil Iluminação, São Paulo, SP, Brazil) and were observed weekly up to 4 mo to detect and record germination events. After 4 mo of culture *in vitro*, final germination percentages were recorded. The germination criterion adopted was the development of normal seedlings (those presenting coleoptile, main root, first leaf, and leaf sheath) as described by Bernacci *et al.* (2008).

**Statistical analysis** All experiments were carried out with three replicates for each treatment. To assess the effects of different water contents and freezing on germination of ZE, a binomial distribution was made using generalized linear models (GLMs) to generate an analysis of deviance table (ANODEV). When the ANODEV factors were significant, the unfolding of degrees of freedom was performed by orthogonal contrasts to obtain comparisons of interest (McCullagh and Nelder 1989). All analyses were conducted using the statistical language program R by GLM function, at a 5% significance level. The statistical programs GraphPad Prism (©2017 GraphPad Software Inc.) and R (free access) were used for the statistical analysis and graphing of the data.

## Results and Discussion

In order to establish a cryopreservation technique for queen palm *S. romanzoffiana*, endocarps, at four levels of water content, were stored in LN and then thawed for ZE excision. The effects of LN storage and thawing on germination and subsequent *in vitro* seedling development were studied.

The fresh fruits were oval shaped and 25 mm or less in length and with an average diameter of 21 to 22 mm (Fig. 1*A*). The fleshy mesocarp was removed to reveal the hard endocarp which generally contains a single seed (Fig. 1*B*). Queen palm seeds are curved, have a tegument lining the endosperm, and are attached to the endocarp by a pronounced recess, which makes their removal without damage very difficult (Fig. 1*C*). Embryos are typically cylindrical, white, and very tender and measure 4.0 to 5.0 mm on average in length and about 1 mm in width (Fig. 1*D*). Handling small and delicate ZE such as these increases the possibility of mechanical injury, compromising their integrity and capacity for growth (Pritchard *et al.* 2000). For this reason, the initial plan was to use whole seeds as the experimental unit for this study, as has been done for other palm species (Engelmann *et al.* 1995). However, extraction of whole seeds from the woody endocarps proved very challenging, and most seeds were crushed in the attempt. Previous studies reported that queen palm endocarps are water permeable and that seeds are able to gain or lose water even inside the endocarps (Baskin and Baskin 2014; Oliveira *et al.* 2015). For this reason, whole endocarps were used as the experimental unit in all the tests carried out in this study. This choice also simplified the procedure, which makes it more practical for routine use in gene banks.

Water content is a critical factor in the maintenance of plant cell viability and integrity after cryopreservation, since high levels of water in the cells will result in the formation of destructive ice crystals upon freezing (Reed 2008; Ferrari *et al.* 2016). However, seeds of many palm species show recalcitrant storage behavior and do not tolerate drying or storage at subzero temperatures (Goudel *et al.* 2013). Although the storage behavior of queen palm seeds has not been thoroughly assessed, previous work showed a consistent decline in seed water content through maturation and that seeds can still germinate after desiccation to 12.7% of the initial water content, a level considered lethal for the germination of many palm species. These results indicate that queen palm seeds are tolerant of partial drying and their storage behavior might be classified as intermediate (Brancalion *et al.* 2011; Goudel *et al.* 2013; Oliveira *et al.* 2015).

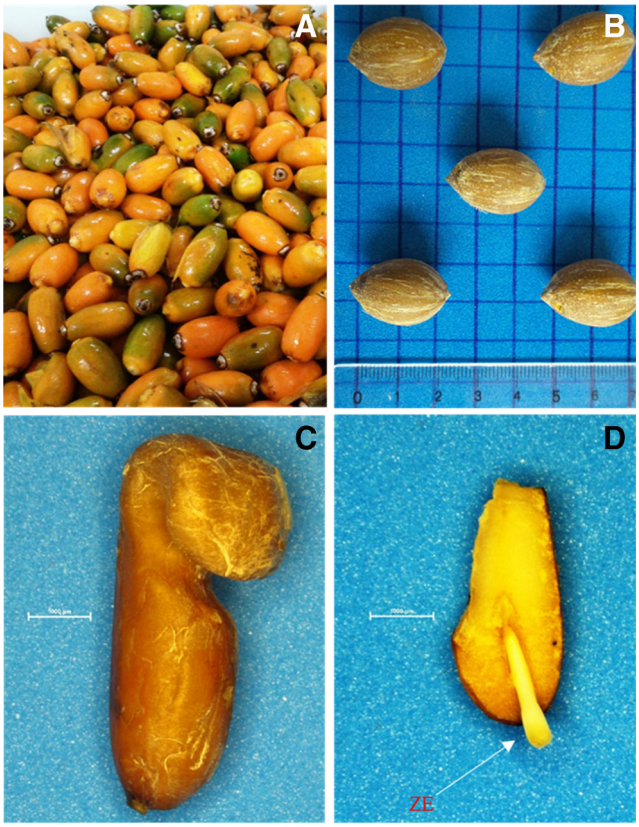


**Figure 1.** Fruit anatomy of the queen palm, *Syagrus romanzoffiana* (Cham.) Glassman. (*A*) Freshly harvested whole fruits. (*B*) Whole endocarp. (*C*) Whole seed excised aseptically from the endocarp. (*D*) Cylindrical zygotic embryo (ZE) still attached to the seed. Whole endocarps (*B*) of different water contents were the structures cryopreserved in liquid nitrogen.

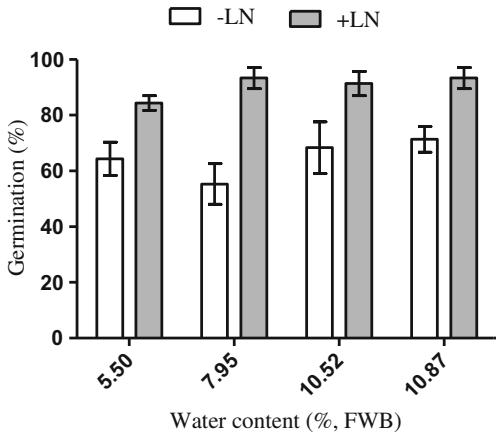


**Figure 2.** *In vitro* germination of zygotic embryos of *S. romanzoffiana* excised from endocarps dried at 25 ± 2°C to different water content (WC) levels and cryopreserved (+LN, *filled*) or not (−LN, *empty*). Values presented are the percentage of ZE germinated on WPM culture medium and represent means ± standard error (SE) of three replicates of 15 embryos. These values only include seedlings with normal morphology, that is, with a plumule, haustorium, and root system and without fungal contamination. WC values presented were calculated on a fresh weight basis (FWB).

The effect of water content on germination of control and cryopreserved ZE was assessed to find an optimal water content to prevent freezing damage. The water content of endocarps was adjusted to 5.5% by dehydration with silica gel or to 10.52 or 10.87% by hydration over 100 mL of distilled water. Silica gel efficiently promoted desiccation of endocarps and caused a marked reduction in WC (Fig. 2). N'Nan *et al.* (2012) pointed out that the use of silica gel ensures more precise and reproducible dehydration, allowing standardization of the method.

The results observed indicate that the WC of the endocarp did not have a significant effect on germination percentages of either control or cryopreserved ZE (Table 1 and Fig. 2). In control endocarps, the germination percentages of the excised ZE ranged from 55 to 71% (Fig. 2, −LN). These results are in agreement with those observed during the assessment of the effect of partial drying on germination of *S. romanzoffiana* seeds (Goudel *et al.* 2013), wherein queen palm seeds dehydrated to 12.7% WC remained viable, showing germination rates, germination speed index, and radicle lengths similar to those of control seeds (20.2% WC). The authors concluded that seeds of this species are more tolerant of partial drying than those of many other palm species. Oliveira *et al.* (2015) also reported that freshly harvested queen palm seeds dried to 5% WC remained viable, although there was a marked decrease in germination after these seeds were stored for 2 y in a cold chamber at 5°C. It is a general view that the significant reductions in viability and vigor of *S. romanzoffiana* seeds are not due to water loss, but rather to deterioration of the seed due to the high lipid content of the endosperm (Goudel *et al.* 2013; Oliveira *et al.* 2015). However, further studies are necessary to elucidate this matter.

Storage in LN caused a significant improvement ($P < 0.001$) in ZE germination (Fig. 2, +LN). The germination percentages of ZE excised from cryopreserved endocarps were higher than the unfrozen controls, ranging from 84 to 93% depending on WC (Fig. 2, +LN). These germination percentages are much higher than the percentages observed after cryopreservation of palm species reported elsewhere. For instance, a comparison of two cryopreservation strategies for oil palm showed that only 25% of ZE excised from whole cryopreserved endocarps remained viable (Engelmann *et al.* 1995). These germination percentages are much lower than the percentages obtained in the present study for queen palm-cryopreserved endocarps. Additionally, the germinated ZEs of queen palm were able to develop into whole, true-to-type plantlets, which was not observed in the study with oil palm. This might be an indication that ZEs of queen palm are more tolerant of desiccation, freezing, or both.

This increase in germination percentages is most likely an indirect effect of freezing. It is possible that the freezing or thawing, and associated changes in vapor pressure, caused micro-fissures in the endocarps, which facilitated hydration

**Table 1.** Significance (5% level) of the variables tested, and of their interaction, on germination percentages and on development of seedlings of *S. romanzoffiana* ZE *in vitro*

| Treatment | Likelihood ratio ($\chi^2$) | Degrees of freedom | $F$ | $P = 0.05$ |
|---|---|---|---|---|
| Cryopreservation | 3978.4 | 1 | 42.3983 | <0.001 |
| Water content | 292.1 | 3 | 1.0377 | 0.4025 |
| Interaction | 307.1 | 3 | 1.0910 | 0.3814 |

Analysis of deviances (ANODEV) obtained from the normal model with logarithmic link function (AIC = 185.3744)

and, subsequently, better cracking with a bench vise during the excision of the ZE. Hydration of both the frozen and control endocarps prior to ZE excision was essential to soften and facilitate breaking them with the vise, thus preventing crushing of the embryo as they ruptured. In a study of the morphogenetic potential of excised palm embryos *in vitro*, Zaid and Tisserat (1984) also reported soaking palm seeds for 48 h to soften them and facilitate embryo excision. Due to endocarp cracking and excision procedures, embryos can suffer mechanical injury, which triggers browning due to oxidation of phenolic compounds and, consequently, reduction in embryo germination percentages (Wen and Wang 2010). A similar effect of LN was proposed during a study of the influence of moisture content on cryopreservation of *Coffea* and *Vigna* seeds and embryos (Normah and Vengadasalam 1992). They hypothesized that storage in LN removed physical dormancy by inducing a network of cracks in or otherwise disrupting the structures surrounding the seed, such as the endocarp (Normah and Vengadasalam 1992), or the embryo, such as the seed coat (Busse 1930; Brant *et al.* 1971). This hypothesis is reinforced by results obtained in a study of other species whose seeds are enclosed by woody endocarps (*Buchenavia tomentosa*, *Byrsonima basiloba*, and *Guettarda pohliana*, and, more remarkably, with *Schinopsis brasiliensis* and *Spondias mombin*), in which the final germination percentages after LN treatment increased in comparison to those of controls (Salomão 2002). Hydration of queen palm endocarps has a significant effect on seed germination, and seeds soaked in water for 24 h before germination tests showed higher scores for normal seedling development, radicle length, and germination speed index (Goudel *et al.* 2013).

Cryopreservation has been reported previously for other palm species, such as *Areca catechu* (Raja *et al.* 2003), *Cocos nucifera* (Chin *et al.* 1989; Assy-Bah and Engelmann 1992), *Bactris gasipaes* (Steinmacher *et al.* 2007), *Elaeis guineensis* (Engelmann *et al.* 1995), *Sabal* spp. (Wen and Wang 2010), and *Veitchia merrilli* and *Howea fosteriana* (Chin *et al.* 1988). Steinmacher *et al.* (2007) reported successful cryopreservation of peach palm ZE using the encapsulation-dehydration method; however, in this case, only 29% of the embryos survived exposure to liquid nitrogen. For four *Sabal* species, pre-incubation combined with dehydration to 18 to 20% WC, direct freezing, and rapid thawing was the best approach to ensure 31 to 44% embryo survival (Wen and Wang 2010). Mature excised embryos of four coconut varieties could withstand cryopreservation in LN and then develop into normal plantlets after air desiccation for 4 h followed by 11 to 20 h of culture on medium with glucose and glycerol, achieving 33 to 93% recovery (Assy-Bah and Engelmann 1992). Raja *et al.* (2003) reported that excised embryonic axes of *A. catechu* with a water content of 21.8% showed 70% survival after cryopreservation. In all these cases, the embryos or embryonic axes were excised before the cryopreservation procedures. Isolated embryo cryopreservation is necessary in many cases to allow better adjustment of water content or to avoid germination difficulties due to dormancy or other problems imposed by the surrounding structures. However, manipulations of isolated embryos may further increase the complexity of and difficulty in the development of a cryopreservation method. Mechanical damage during embryo excision is very likely to occur, and even minor injuries can lead to tissue browning due to oxidation of phenolic compounds (Pritchard *et al.* 2000). Another factor to consider is that excised embryos can be extremely sensitive to desiccation. These occurrences can affect embryo germination and seedling development in culture, compromising the establishment of a cryopreservation protocol. Wen and Wang (2010) observed that cryopreserved embryos of four *Sabal* species that were excised prior to freezing showed lower emergence *in vitro* than the controls and concluded that this possibly resulted from differences in manipulations of the embryos, since preparation for cryopreservation requires extra procedures that cause a delay in embryo culture and might affect their viability.

Seedling emergence, as the marker for germination, was evaluated weekly up to 4 mo to calculate the final germination percentages for *S. romanzoffiana* ZEs (Fig. 1). The majority of seedlings obtained from control and cryopreserved ZE showed normal morphology, with a normal haustorium and roots, and, after 4 mo of culture *in vitro*, had fully expanded, bright green leaves (Fig. 3). As is generally the case for this species, germination of ZE was uneven and slow, even with the excision from the endocarp and growth *in vitro* under stimulating conditions. According to Baskin and Baskin (2014), the primary reason for this is that seeds of palm trees have small and immature embryos that must complete their development before germination can occur. Fruit bunches of queen palm contains fruits in different stages of maturation. It

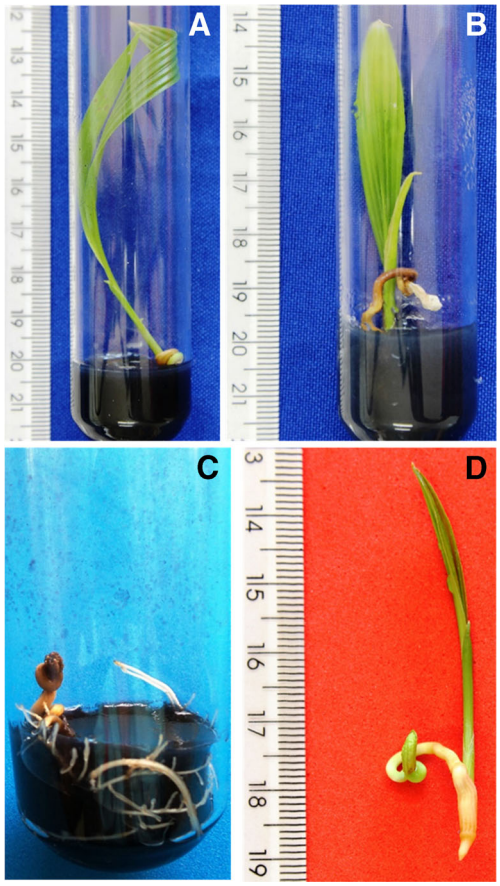


**Figure 3.** Seedlings originated from zygotic embryos of *S. romanzoffiana* germinated *in vitro* on WPM culture medium, after 4 mo of culture. (*A*) Normal seedlings with fully expanded leaves obtained from control zygotic embryos and (*B*) from embryos stored in liquid nitrogen. Abnormal seedlings lacking (*C*) the plumule or (*D*) the root system.

is likely that fruits that are not fully mature are not capable of expressing the full germination potential of the species (Begnini *et al.* 2013; Oliveira *et al.* 2015; Garcia and Barbedo 2016).

Some seedlings showed an abnormal development of the root system or the haustorium (Fig. 2*C*, *D*). Damage to the root system occurred in ZE excised from endocarps containing the lowest water content (5.5%) and was associated with water loss. Damage to the haustorium was observed in ZE excised from cryopreserved endocarps (Fig. 3*D*), as reported elsewhere in studies with other species of palms (Assy-Bah and Engelmann 1992; Steinmacher *et al.* 2007). In the present study, the final germination percentages, represented in Fig. 2, do not include the abnormal seedlings. Some of the ZE at the initial stages of germination *in vitro* showed fungal contamination. These embryos were discarded and were not included in the final germination percentages.

The excision and *in vitro* culture of ZE is a practical method for recovering cryopreserved seeds that might otherwise exhibit germination difficulties under laboratory conditions. The present study reports on a simple cryopreservation technique for *S. romanzoffiana*, involving partial desiccation of endocarps followed by direct freezing, slow thawing, and aseptic excision and culture *in vitro* of ZE. Excision and *in vitro* culture of embryos after cryopreservation is a simple technique that has been applied to many other plant species including *C. nucifera* (Assy-Bah and Engelmann 1992), *V. merrilli* and *H. fosteriana* (Chin *et al.* 1988), and *Genipa americana* (Santos and Salomão 2016), and it has been helpful in the assessment of viability and the regeneration of plantlets from cryopreserved seed samples (Pritchard *et al.* 2000).

Queen palm ZE excised from endocarps with different water contents (5.5, 7.95, 10.52, and 10.87%) were successfully cryopreserved, with high germination percentages ranging from 84 to 93%. Cryopreservation in LN was the only treatment with a significant effect on the germination percentages of ZE, at the 0.05 probability level. The technique described here is simple and efficient, since a high percentage of cryopreserved ZE retained capacity to germinate and develop into whole, normal plantlets. However, to improve and validate this procedure, additional trials should be performed with larger numbers of endocarps, with endocarps from other regions and genetic backgrounds, and with endocarps at different maturation stages.

## Conclusions

The results obtained in the present work provide novel evidence that cryopreservation is a feasible choice for the preservation of *S. romanzoffiana* germplasm to ensure long-term *ex situ* conservation of this species. This appears to be the first report of the cryopreservation of queen palm endocarps and *in vitro* rescue of the ZEs of this species.

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