Plant Cell Culture & Micropropagation

http://pccm.ufla.br/index.php/plantcellculturemicropropagation/index

In vitro germination of *Syagrus romanzoffiana* (Cham.) Glassman

Aurélio Rubio Neto[1*], Caroline De Araujo Machado[2], Edvan Alves Chagas[3], Barbara Nogueira Souza Costa[4], Pollyana Cardoso Chagas[5], Wagner Aparecido Vendrame[6]

ABSTRACT

Syagrus romanzoffiana (Cham.) Glassman belongs to the Arecaceae family and is native to South America, with large importance as an ornamental plant. Therefore, the objective of this study was to evaluate TIS bioreactors for *in vitro* germination of queen palm. Traditional (agar-based) *in vitro* and TIS bioreactor technologies were compared. We evaluated parameters for the optimization of the tetrazolium test for seed viability and a protocol to increase germinated seedling survival after *in vitro* propagation. Fruits of *S. romanzoffiana* were collected in October 2017 from 5 individual trees located at the Tropical Research and Education Center, University of Florida. A total of 40 embryos were established in each culture system. The culture medium was refreshed every 30 days. Four replicates of 7 embryos were subjected to the tetrazolium test under different concentrations. Embryos were transferred to either a temporary immersion system in SETIS bioreactors using liquid medium or to test-tubes containing an agar-based semi-solid medium. After 45 days under *in vitro* conditions, germinated embryos were carefully removed from both the agar and liquid media, thoroughly washed and transferred to *ex vitro* conditions using small plastic pots. We have concluded that the TTC test can be successfully used to determine seed viability in *S. romanzoffiana* regardless of the TTC concentration evaluated in our study (0.25 to 1%). The traditional semi-solid *in vitro* system showed to be more effective for *in vitro* queen palm zygotic embryo germination compared to the TIS system. Seedlings from both systems showed similar growth characteristics.

Index terms: Queen palm; bioreactors; embryo.

INTRODUCTION

The queen palm, *Syagrus romanzoffiana* (Cham.) Glassman belongs to the Arecaceae family and is native to South America, with large importance as an ornamental plant. This palm is also utilized in other continents, especially in subtropical and tropical regions, including south Florida in the United States (Lorenzi et al., 1996, Iossi et al., 2016), but it has great potential to adapt to different environment conditions (Oliveira et al., 2015b). In addition to its ornamental potential, queen palms are reported to have economic importance due to their oil content and nutritional value of seeds (Coimbra; Jorge, 2011; Lescano et al., 2018). A recent study with queen palm suggests the pulp can be used as an agar substitute in *in vitro* culture (Assis et al., 2018).

The queen palm became very popular in United States due to some major diseases in *Cocos nucifera* in the 70's, which created the need for new palm species (Elliott et al., 2010). Queen palms are propagated exclusively by seeds. However, despite its economic importance, studies on commercial production are limited to seed germination (Cintra et al., 2003), seed dormancy (Goudel et al., 2013), and seed ripening and storage (Oliveira et al., 2015a).

In vitro embryo germination has been used in palms to assist with plant production and seed conservation especially in species with seed dormancy or recalcitrant seeds. Some studies have

https://doi.org/10.46526/pccm.2020.v16.161
Received in September 17, 2020 and approved in December 28, 2020
[1]Instituto Federal Goiano/IF Goiano, Goiania, GO, Brasil
[2]Universidade Federal de Sergipe/UFS, São Cristovão, SE, Brasil
[3]Empresa Brasileira de Pesquisa Agropecuária/Embrapa, Embrapa Roraima, Boa Vista, RR, Brasil
[4]Universidade Federal de Lavras/UFLA, Lavras, MG, Brasil
[5]Universidade Federal de Roraima/UFRR, Boa Vista, RR, Brasil
[6]University of Florida, Environmental Horticulture Department, Institute of Food and Agricultural Sciences, Florida, EUA
*Corresponding author: e-mail: aurelio.rubio@ifgoiano.edu.br

been reported using tetrazolium tests in traditional *in vitro* germination and for *in vitro* conservation of plant material (Pritchard; Beeby; Davies, 2000; Ribeiro et al., 2010; Sarmento et al., 2013; Oliveira et al., 2014). Seed viability with tetrazolium tests on queen palm embryos was reported in two individual studies looking at different imbibition times and salt concentration, respectively (Oliveira et al., 2015a; Iossi et al., 2016). However, *in vitro* germination studies and protocol development to increase the queen palm production are scarce.

As an alternative for traditional *in vitro* culture, temporary immersion systems (TIS) using liquid culture in bioreactors have been used to produce plants, including several prototypes that could improve *in vitro* photomixotrophic conditions and therefore increase plant production (Arencibia et al., 2017), as well as produce plants that are more adaptive to acclimatization *ex vitro.* Bioreactor technology could represent a significant progress for the industrial development of clonal propagation in queen palm as compared with the traditional micropropagation (Zhang et al., 2018). Therefore, the objective of this study was to evaluate TIS bioreactors for *in vitro* germination of queen palm. Traditional (agar-based) *in vitro* and TIS bioreactor technologies were compared. We evaluated parameters for the optimization of the tetrazolium test for seed viability and a protocol to increase germinated seedling survival after *in vitro propagation.*

MATERIAL AND METHODS

Plant material

Fruits of queen palm, *Syagrus romanzoffiana* (Cham.) Glassman were collected in October 2017 from 5 individual trees located at the Tropical Research and Education Center, University of Florida, in Homestead, Florida, United States (N 25°28.8551', W 80°25.5230', 10m). All fruits were homogenized based on size and epicarp color (green, yellow and orange), and orange fruits were selected for the experiments. Damaged fruits were discarded. Fruits were surface disinfected by immersion in sodium hypochlorite solution (3% active chlorine, Clorox©) for 48 hours. After 48 hours in room temperature (25 ± 3 °C), the pulp was removed from fruits. The diaspores (seed + endocarp) were maintained in room temperature (25 ± 3° C) for 12 days for drying in order to facilitate seed extraction.

Water content determination

The water content of seeds was determined at harvesting and at 12 days after drying. Seeds from 15 fruits were placed in an oven at 105 ± 1 °C for 24 hours for water content evaluation (Brasil, 2009).

Zygotic embryo excision and tetrazolium test

Diaspores (seed and endocarp) were ruptured to extract the seeds and embryos were removed from seeds with a scalpel. Four replicates of 7 embryos were subjected to the tetrazolium test under different concentrations, following the same criteria as in Oliveira et al. (2015) and Iossi et al. (2016). Evaluations included embryo immersion on 2,3,5-triphenyl-tetrazolium chloride (TTC) at 0.25%, 0.50%, 0.75% and 1.0% for 6 hours at 40 ± 1 °C in the dark.

In vitro germination

After extraction, embryos were placed in a 500-mL flask with autoclaved water and 0.5 $g.L^{-1}$ citric acid. Disinfection involved covering the embryos with a gauze followed by immersion in ethanol 70% for 30s, sodium hypochlorite 3% for 15 min and rinsing three times with sterile water for 3 min each under constant agitation. Embryos were transferred to either a temporary immersion system in SETIS bioreactors (Vervit, Belgium) using liquid medium or to test-tubes containing an agar-based semi-solid medium. For both culture systems, MS (Murashige; Skoog, 1962) was the culture medium used, supplemented with 2% active charcoal (Sigma-Aldrich, St. Louis, MO, USA) and 2.0 g. L^{-1} Phytagel (Sigma-Aldrich, St. Louis, MO, USA) for semi-solid MS and 2% polivinylpyrrolidone (Sigma-Aldrich, St. Louis, MO, USA) for liquid MS. A total of 40 embryos were established in each culture system. The culture medium was refreshed every 30 days. For both *in vitro* systems, embryos were kept for 45 days in growth room at 26 ± 2 °C in the dark for 7 days (and then under 12 hours photoperiod with 70 $\mu M.m^{-2}.s^{-1}$).

After this period, embryos germinated under traditional *in vitro* culture were divided into two groups with 20 embryos each. The first group was kept in traditional *in vitro* culture while the second

group was transferred to the TIS bioreactors to evaluate growth characteristics. For embryo cultures in TIS bioreactors, parameters included immersion in liquid MS medium every 240 min with duration of 60s for each immersion, and aeration every 480 min for 20s. Germination was recorded daily and the germination speed index was calculated using the formula suggested by (Maguire, 1962). Five replications of 10 zygotic embryos each were utilized.

Seedling Acclimatization

After 45 days under *in vitro* conditions, germinated embryos were carefully removed from both the agar and liquid media, thoroughly washed and transferred to *ex vitro* conditions using small plastic pots (6 inches) containing Pro-Mix BX substrate (Quakertown, PA, USA). Pots containing seedlings were covered with a plastic bag for 21 days, using shade cloth in a greenhouse with manual irrigation. After 7 days one cut was made in the edge of the plastic bag, followed by an additional cut at 14 days, and removal of plastic bag at 21 days. A total of 20 seedlings from each *in vitro* culture system were evaluated for survival.

Statistical analysis

The experiment was conducted under a completely randomized design. Data was submitted to the Shapiro-Wilk normality test with 5% probability, followed by the Kruskal Wallis test under 5% probability level to detect any difference between treatments.

RESULTS AND DISCUSSION

The average seed water content was 17% (17.01 ± 0.53) at harvesting and 6% (6.08 ± 0.09) at 12 days after drying. Embryos extracted from these seeds reached 70% germination after 30 days under *in vitro* conditions. This germination under low water content suggests an orthodox behavior for the seeds in this species, although biochemical analyses would be necessary to confirm this behavior (Oliveira et al., 2015a).

Regardless of the tetrazolium concentration used, there were no significant differences for embryo viability tests (*p-value>0.05*). Over 60% of the embryos were viable, showing pink/red color on all proximal regions of petiole for all tetrazolium concentrations used. *In vitro* germination correlated with viability tests, showing that the use of 2,3,5-triphenyl tetrazolium chloride (TTC) concentrations between 0.25 and 1% are efficient to evaluate the viability of queen palm seeds (Figure 1). The pro-meristem in queen palm seeds is located in the proximal region of petiole. Therefore, the color change to red induced by the TTC test is an indication of success in germination. Otherwise, if no color change occurred at the haustorium region (no damage at haustorium area) the germination process can still proceed under *in vitro* conditions.

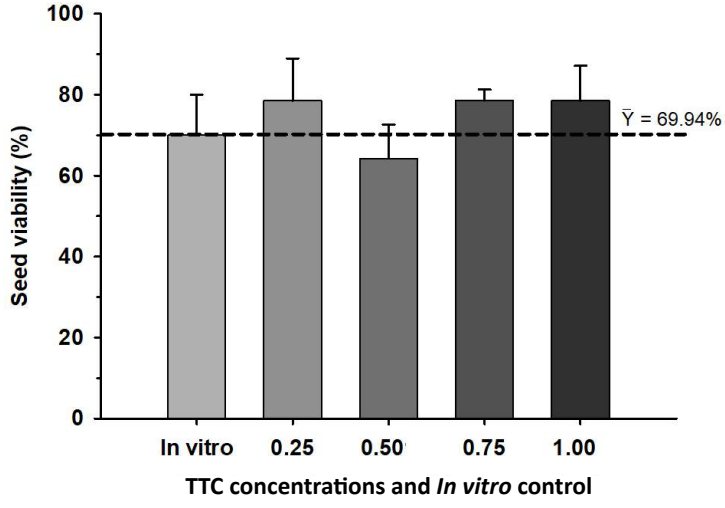

Figure 1: Viability of *Syagrus romanzoffiana* (Cham.) Glassman seeds using different concentrations of 2,3,5-triphenyl chloride tetrazolium (TTC). The control consisted of *in vitro* germination of seeds, evaluated as percent germination. Bar = standard error. Ȳ = average.

This is confirmed by Iossi et al. (2016) who reported similar results on viability in queen palm seeds for both areas, the haustorium and the proximal region of petiole. This suggests that the most important region for the germination process is the proximal region of the petiole (Figure 2). The haustorium showed positive results only when using 1% TTC solution suggesting that the haustorium region was either dead or that salts penetrated the tissue, as suggested by Iossi et al. (2016).

However, this did not interrupt *in vitro* germination process. Thus, it is possible that the low level of water content at 14 days could have caused embryo deterioration (chemical deterioration), as suggested by Oliveira et al. (2015a). There are two tetrazolium papers with this specie with different tetrazolium concentration and imbibitions time. In this study we considered previous reports (Oliveira et al., 2015a; Iossi et al., 2016) as the basis for our *in vitro* experiment and demonstrated that any TTC concentration would be feasible for *in vitro* germination, being able to differentiate between viable and non-viable embryos. Regardless of TTC concentration, less than 20% of non-viable and dead embryos were observed (Figure 3).

We have compared the traditional semi-solid *in vitro* method with a new liquid medium temporary immersion bioreactor system (TIS). The use of liquid systems brings a potential cost reduction for *in vitro* propagation by eliminating the need for agar. In addition, there are physiological advantages that can increase survival in the field (Gomes et al., 2016). However, in our studies, after 15 days in TIS, the proximal region of petiole in the embryos showed a color change from white to brown/black. After 30 days in TIS, embryos did not show any germination. The germination percentage of 70% (69.94 ± 17.89) was obtained using the traditional semi-solid medium with a germination speed index (GSI) of 0.2 (0.1906 ± 0.08). New immersion frequencies and times, as well as liquid medium composition should be evaluated for success using TIS.

Germinated seedlings were subsequently divided into two groups and placed into cultivation on both traditional and TIS systems. No significant differences were observed among growth characteristic averages between both systems, including height (2.74 ± 0.21cm), root number (0.83 ± 0.07), and leaf number (1.72 ± 0.12) (Table 1).

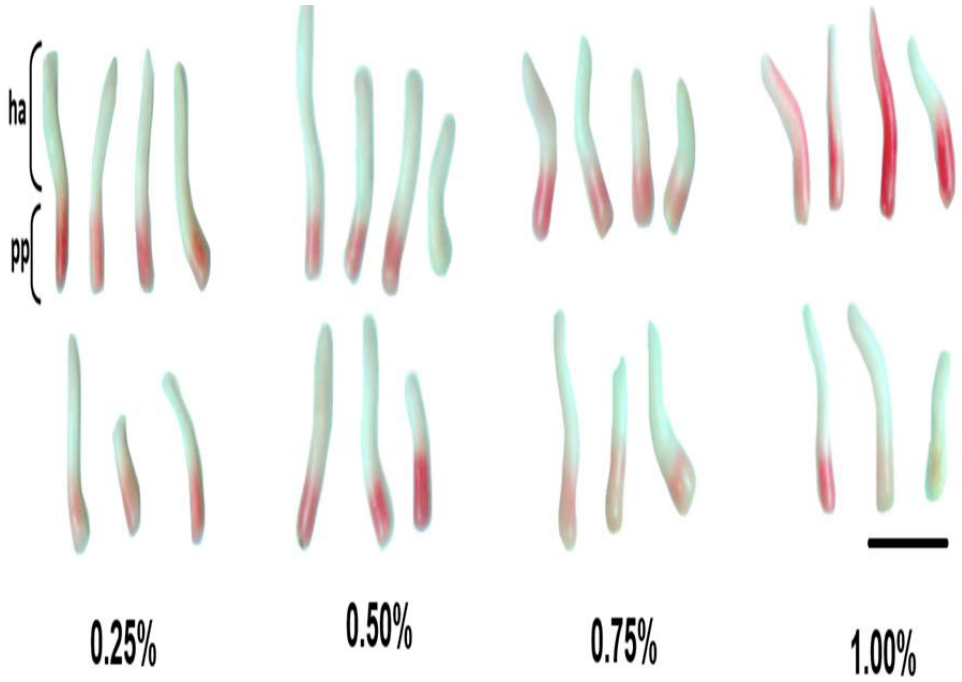

Figure 2: *Syagrus romanzoffiana* (Cham.) Glassman embryos submitted to the 2,3,5-triphenyl chloride tetrazolium (TTC) test under different concentrations for 6 hours at 40 °C in the dark. Ha = haustorium; pp = proximal region of petiole. Bar = 1cm.

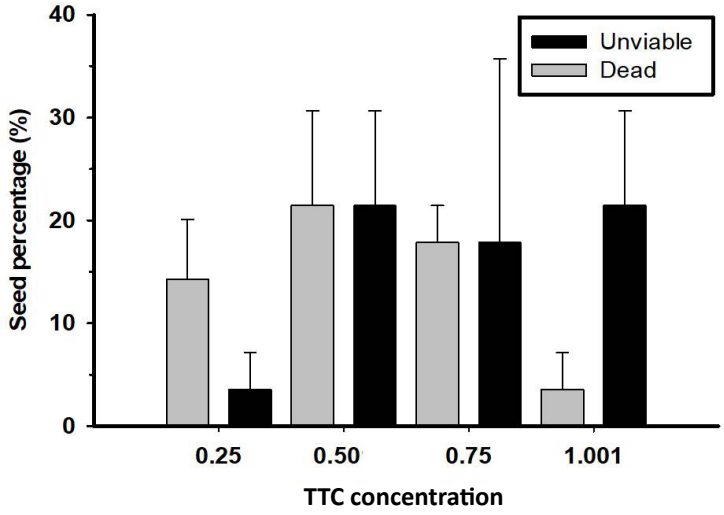

Figure 3: Viability for non-viable and dead zygotic embryos of *Syagrus romanzoffiana* (Cham.) Glassman under different 2,3,5-triphenyl tetrazolium chloride (TTC) concentration test. Bar = standard error.

Table 1: *Syagrus romanzoffiana* (Cham.) Glassman seedling growth characteristics and survival after 50 days of cultivation on semi-solid culture medium (traditional) and temporary immersion bioreactor system (TIS).

In vitro conditions	Height (cm)	Number of leaves	Number of roots	Survival (%)
Traditional	2.86	1.93	0.93	71.4
TIS	2.63	1.53	0.73	0
Average	2.74	1.73	0.83	35.7
p-value	0.4206^{ns}	0.1281^{ns}	0.1717^{ns}	0.0001*

^{ns}Non-significant.
*Significantly different by Kruskal-Wallis test at 5% probability.

Seedlings obtained from both systems were transferred to the greenhouse for acclimatization and evaluation of survival. After 30 days in the greenhouse we observed no survival in seedlings derived from TIS, while 71.4% survival was observed in seedlings derived from traditional semi-solid *in vitro* system (Table 1). The utilization of TIS systems in palms are quite limited, including the use of RITA systems for *in vitro* multiplication of embryogenic callus in *Bactris gasipaes* Kunth, *Phoenix dactylifera* L. and *Euterpe edulis* Mart (Fki et al., 2011; Steinmacher et al., 2011; Heringer et al., 2014; Gomes et al., 2016). However, no reports exist for queen palm.

CONCLUSIONS

TTC test can be successfully used to determine seed viability in *Syagrus romanzoffiana* regardless of the TTC concentration evaluated in our study (0.25 to 1%). *In vitro* germination is warranted provided that the proximal region of petiole in the embryo dies either pink or red through the TTC test. The traditional semi-solid *in vitro* system showed to be more effective for *in vitro* queen palm zygotic embryo germination compared to the TIS system. Seedlings from both systems showed similar growth characteristics. However, seedling survival in the greenhouse was only achieved under the traditional *in vitro* system.

This is the first report to attempt the use of a temporary immersion bioreactor system for zygotic embryo germination and initial cultivation of *Syagrus romanzoffiana* (Cham.) Glassman. Our study suggests that adjustments for the *in vitro* protocol are necessary for *in vitro* germination of seeds and to improve seedling survival in queen palm.

REFERENCES

ARENCIBIA, A. D. et al. High-performance micropropagation of dendroenergetic poplarhybrids in photomixotrophic Temporary Immersion Bioreactors (TIBs). **Industrial Crops and Products**, 96:102-109, 2017.

BRASIL. Ministério da Agricultura, Pecuária e Abastecimento. **Regras para análise de sementes**. Brasília, DF: Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária, 2009. 395p.

CINTRA, G. et al. Effect of temperature on seed germination of queen palm *Syagrus romanzoffiana* (Cham.) *Glassman* (Arecaceae). In: **V International Symposium on New Floricultural Crops**, 683:379-382, 2003.

COIMBRA, M. C.; JORGE, N. Proximate composition of guariroba (Syagrus oleracea), jerivá (*Syagrus romanzoffiana*) and macaúba (*Acrocomia aculeata*) palm fruits. **Food Research International**, 44(7):2139-2142, 2011.

ELLIOTT, M. et al. *Fusarium oxysporum f. sp. palmarum*, a novel forma specialis causing a lethal disease of *Syagrus romanzoffiana* and *Washingtonia robusta* in Florida. **Plant Disease**, 94(1):31-38, 2010.

FKI, L. et al. Multiple bud cultures of 'Barhee'date palm (*Phoenix dactylifera*) and physiological status of regenerated plants. **Journal of Plant Physiology**, 168(14):1694-1700, 2011.

GOMES, H. T. et al. Regeneration of somatic embryos of oil palm (*Elaeis guineensis*) using temporary immersion bioreactors. **Industrial Crops and Products**, 89(1):244-249, 2016.

GOUDEL, F. et al. Fruit biometry and seed germination of *Syagrus romanzoffiana* (Cham.) Glassm. **Acta Botanica Brasilica**, 27(1):147-154, 2013.

HERINGER, A. S. et al. Improved high-efficiency protocol for somatic embryogenesis in Peach Palm (*Bactris gasipaes Kunth*) using RITA® temporary immersion system. **Scientia Horticulturae**, 179(1):284-292, 2014.

IOSSI, E. et al. Chemical composition and tetrazolium test of *Syagrus romanzoffiana* (Cham.) Glassman seeds. **Revista Brasileira de Fruticultura**, 38(4):1-10, 2016.

LESCANO, C. H. et al. Nutritional and chemical characterizations of fruits obtained from *Syagrus romanzoffiana*, *Attalea dubia*, *Attalea phalerata* and *Mauritia flexuosa*. **Journal of Food Measurement and Characterization**, 12(2):1-11, 2018.

LORENZI, H. et al. Palmeiras no Brasil: Nativas e exóticas. **Nova Odessa: Plantarum**, 1996. 303p.

MAGUIRE, J. D. Speed of germination-aid in selection and evaluation for seedling emergence and vigor. **Crop Science,** 2(2):176-177, 1962.

OLIVEIRA, T. et al. Seed germination of queen palm (*Syagrus romanzoffiana*) is affected by storage and fruit ripening stage. **Seed Science and Technology**, 43(3):399-408, 2015a.

OLIVEIRA, T. G. S. et al. Longevity and germination of *Syagrus romanzoffiana* (Arecaceae) seeds and its ecological implications. **Revista de Biologia Tropical**, 63(2):333-340, 2015b.

OLIVEIRA, L. M. et al. Viability evaluation of *Euterpe edulis* seeds by the tetrazolium test. **Magistra**, 26(3):403-411, 2014.

PRITCHARD, H. W.; BEEBY, L. A.; DAVIES, R. I. Role of embryo culture in the seed conservation of palms and other species. In: RAZDAN, M. K.; COCKING, E. C. (Eds). **Conservation of plant genetic resources *in vitro*.** Volume 2: Applications and limitations. Science Publishers Inc, Barking, p.89-138, 2000.

RIBEIRO, L. M. et al. Criteria for the tetrazolium test in the estimation of the germinative potential in macaw palm**. Pesquisa Agropecuária Brasileira**, 45(4):361-368, 2010.

SARMENTO, M. B. et al. Tetrazolium test to evaluate physiological quality of brazilian guava seeds (*Acca sellowiana* O. Berg Burret). **Revista Brasileira de Fruticultura**, 35(1):270-276, 2013.

STEINMACHER, D. et al. A temporary immersion system improves *in vitro* regeneration of peach palm through secondary somatic embryogenesis. **Annals of Botany**, 108(8):1463-1475, 2011.

ZHANG, B. et al. Optimizing factors affecting development and propagation of *Bletilla striata* in a temporary immersion bioreactor system. **Scientia Horticulturae**, 232:121-126, 2018.