



Preliminary Studies on In Vitro Propagation of Camu-Camu (*Myrciaria dubia*), an Important Medicinal Plant

MARIA C.R. ARAÚJO¹, WAGNER A. VENDRAMÉ*², EDVAN A. CHAGAS³,
MARIA IZABEL GARCIA RIBEIRO¹, AND ROSEMARY VILACA⁴

¹*Universidade Federal de Roraima, Boa Vista, RR, Brazil*

²*University of Florida, IFAS, Tropical Research and Education Center, Environmental Horticulture,
18905 SW 280th St., Homestead, FL 33031*

³*Embrapa CPAFRR Roraima, Departamento de Fruticultura, Boa Vista, RR, Brazil*

⁴*Embrapa CPAFRR Roraima, Departamento de Biologia Molecular, Boa Vista, RR, Brazil*

ADDITIONAL INDEX WORDS. micropropagation, plant tissue culture, somatic embryogenesis

Camu-camu (*Myrciaria dubia*) belongs to the Myrtaceae family and is a small tree, found on the margins of rivers and lakes, which is native to the Amazon region. This species has great nutritional value, mainly due to the high levels of potassium and ascorbic acid (vitamin C), with values ranging from 1000–6000 mg/100 g in its pulp. In spite of its economic importance, little is known about the aspects of propagation of camu-camu, particularly in vitro multiplication, which could significantly increase yields. Therefore, the objective of this study was to develop a feasible system for in vitro multiplication of camu-camu using somatic embryogenesis. Different concentrations of a cytokinin and an auxin in two different media were evaluated. Embryogenic callus was induced from leaf and stem explants on either Murashige and Skoog (MS) or Woody Plant Media (WPM) medium, supplemented with various concentrations of 2,4-dichlorophenoxy acetic acid (2,4-D) (0, 1.0, 2.0, and 4.0 mg·L⁻¹) along with Benzylaminopurine (BAP) (0, 0.25, 0.5, and 1.0 mg·L⁻¹). Preliminary results showed induction of embryogenic tissues on WPM medium with higher concentrations of 2,4-D and BAP, and using stem segments as the source of explants.

Camu-camu (*Myrciaria dubia* (H.B.K.) McVaugh) is a small fruit tree native to the Amazon region. It is a member of the Myrtaceae family that grows on the margins of rivers and lakes throughout the Amazon basin. The camu-camu fruit is considered of high nutritional value, especially because of its high levels of ascorbic acid, but it also contains high levels of potassium, vitamin A, glucose, fructose, starch, pectin, phosphorus, nitrogen, and various bioactive compounds such as phenolic compounds and carotenoids. (Yuyama, 2011; Akachi et al., 2010; Zanatta and Mercadante, 2007; Silva et al., 2006; and Zanatta et al., 2005).

This fruit has great economic value because it contains an impressive quantity of vitamin C, ranging from 845–6112 mg/100 g in its pulp. This makes it one of the richest sources of vitamin C in the world (Yuyama et al., 2002). Camu-camu is not consumed out of hand due to its high acidity. Consequently, the pulp is processed and is widely used in soft drinks, ice cream, jelly, liquor, syrup, shampoo, yogurt, extracts, and juice, which

are mainly exported to Japan and Europe, or as an ingredient in the production of several food products (Akter et al., 2011; Castañeda et al., 2008; Chirinos et al., 2010; Rodrigues et al., 2004; and Teixeira et al., 2004).

Camu-camu is commonly propagated by seeds, which come to full maturity after fruit ripening. Germination starts in 12 days reaching 90% at 50 days after seed production (Yuyama et al., 2011). Despite the high viability, seeds are not adequate for commercial plantations due to the high genetic diversity. Therefore vegetative propagation methods are necessary for the clonal propagation and commercial production of uniform plants (Chagas et al., 2012).

Plant tissue culture techniques offer viable methods for the in vitro clonal propagation (micropropagation) of several fruit species, providing homogeneous populations of plants faster than conventional propagation methods (Souza et al., 2007). Micropropagation allows year-round production of large numbers of genetically identical plants (Schuch and Erig, 2005).

Somatic embryogenesis is a viable technique for in vitro propagation and regeneration of tree species, allowing the production of plants from a single progenitor cell and thereby maintaining clonal identity and avoiding chimeras (Poupin and Arce-Johnson, 2005). To date, no studies have been reported on in vitro propagation of camu-camu.

Acknowledgments. The authors wish to thank CAPES for the scholarship support provided for Mrs. Araújo's to perform this study.

*Corresponding author. Email: vendrame@ufl.edu

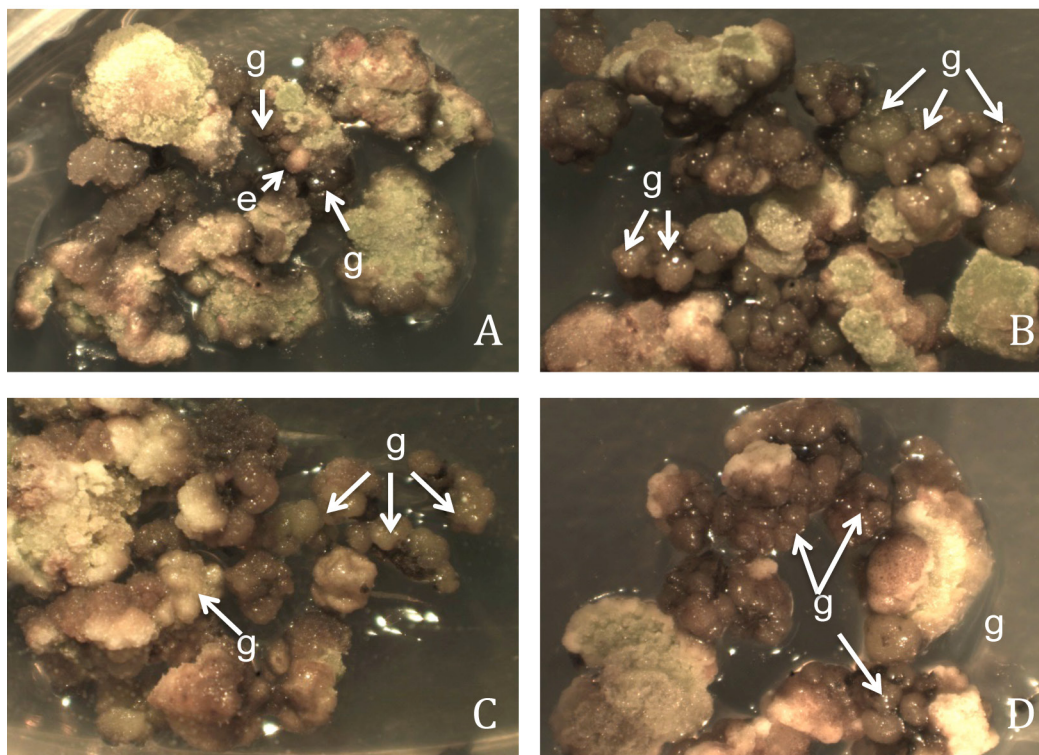


Fig. 1. Camu-camu (*Myrciaria dubia*) pro-embryogenic mass (PEM) formation in stem explants under various treatments with different concentrations of 2,4-D and BAP. (A) PEM formation with 4.0 mg·L⁻¹ 2,4 D + 1.0 mg·L⁻¹ BAP in WPM medium. Small globular embryos (g) are present with one apparently elongated embryo (e). (B) PEM formation is higher, with large globular embryos (g) as BAP concentration increases; 4.0 mg·L⁻¹ 2,4 D + 0.25 mg·L⁻¹ BAP in MS medium. (C) PEM formation and globular embryo (g) presence with 4.0 mg·L⁻¹ 2,4 D + 1.0 mg·L⁻¹ BAP in MS medium. (D) Lower PEM formation with fewer globular embryos (g) with 1.0 mg·L⁻¹ 2,4 D + 0.5 mg·L⁻¹ BAP in WPM medium, as compared to other treatments.

Therefore, this study aims at the development of a feasible system for in vitro propagation of camu-camu using somatic embryogenesis. Different concentrations of a cytokinin and an auxin in two different media are evaluated.

Materials and Methods

Seeds of camu-camu (*Myrciaria dubia*) were obtained from the germplasm collection at Embrapa Roraima's research center in Roraima, Brazil and germinated in the greenhouse at University of Florida's Tropical Research and Education Center (TREC) in Homestead, FL. Three months after germination, two different explant types (leaf and stems sections) were collected and disinfested as follows: 10 minutes in 1% Alconox solution, followed by transfer to a laminar flow hood, where the explants were disinfested with 8% sodium hypochlorite for 1 minute followed by three 3-minute rinses in sterilized distilled water. Afterwards, explants were placed in a petri dish containing either Murashig and Skoog (MS) or Woody Plant Media (WPM) media solidified with 7 g·L⁻¹ agar and containing 30 g·L⁻¹ sucrose, 100 mg·L⁻¹ myo-inositol, 100 mg·L⁻¹ casein hydrolysate, and different combinations of 2,4-D (0, 1.0, 2.0, and 4.0 mg·L⁻¹) and BAP (0, 0.25, 0.5, and 1.0 mg·L⁻¹) concentrations. The media pH was adjusted to 5.7 before autoclaving at 121 °C for 30 minutes at 1.2 kg·cm⁻².

After 30 days, explants were transferred to fresh media for maintenance and callus multiplication. Cultures were maintained in the dark at 25 °C for 60 days, after which they were transferred to a 2,4-D free medium.

The experimental design was completely randomized in a factorial with 2 culture media x 4 concentrations of 2,4-D x 4 concentrations of BAP. Each treatment consisted of five petri dishes containing 5 explants each, totaling 32 treatments and 800 explants.

Results and Discussion

To date tissues similar to pro-embryogenic masses (PEMs) and callus were observed under different treatments. The highest percentage of PEMs was observed in treatments with higher 2,4-D concentrations (4 mg·L⁻¹) combined with any BAP concentrations (Fig. 1A, 1B, 1C).

The lowest percentage of PEMs and callus formation was observed in treatments with lower 2,4-D concentrations or with BAP alone at all different concentrations (Fig. 1D).

PEMs had a round smooth surface, similar to those of globular embryos during the process of somatic embryogenesis (Fig. 1A, 1B, 1C). In certain treatments, these structures seemed to be slightly elongated, similar to torpedo stages of somatic embryos (Fig. 1A). In other treatments PEMs were more prolific and larger in size (Fig. 1B, Fig. 1C). However, in treatments with low or no 2,4-D, PEMs seemed darker in color and smaller in size (Fig. 1D). Stem sections appeared to be the best explants for the induction of PEMs, which can be an indication that this type of tissue is more amenable for the micropropagation of camu-camu via somatic embryogenesis.

No differences were observed in callus and PEM formation when comparing either MS or WPM medium under all different

treatments, suggesting the effect of callus or PEM formation is mostly induced by the combination of auxin (2,4-D) and cytokinin (BAP) in different concentrations.

Studies continue in order to elucidate mechanisms of somatic embryo elongation, maturation and plant regeneration. The present study is the first to report the formation of pro-embryogenic masses in camu-camu, serving as a precursor for the development of a viable in vitro propagation system for this species via somatic embryogenesis.

Literature Cited

- Akachi, T., S. Yasuyuki, T. Kawaguchi, M. Tatsuya, and K. Sugiyama. 2010. 1-methylmalate from camu-camu (*Myrciaria dubia*) suppressed D-galactosamine-induced liver injury in rats. *Biosci. Biotechnol. Biochem.* 74:573–578.
- Akter, M.S., S. Oh, J.B. Eun, and M. Ahmed. 2011. Nutritional compositions and health promoting phytochemicals of camu-camu (*Myrciaria dubia*) fruit: A review. *Food Res. Intl.* 44(7):1728–1732.
- Castañeda, C.B., Q.F. Ramos, and V.L. Ibáñez. 2008. Evaluation of the antioxidant capacity of seven Peruvian medicinal plants. *J. Med. Hor.* 8:56–78.
- Chagas, E.A., C.G., Bacelar-Lima, A.S. Carvalho, M.I.G. Ribeiro, R.T. Sakazaki, and L.C. Neves. 2012. Propagação do camu-camu (*Myrciaria dubia* (H.B.K.) Mcvaugh). *Agro@mbiente Online* 6:67–73.
- Chirinos, R., J. Galarza, I. Betallez-Pallardel, R. Pedreschi and D. Campos. 2010. Antioxidant compounds and antioxidant capacity of Peruvian camu camu [*Myrciaria dubia* (H.B.K.) McVaugh] fruit at different maturity stages. *Food Chem.* 120(4):1019–1024.
- Poupin, M.J. and P. Arce-Johnson. 2005. Transgenic trees for a new era. *In Vitro Cell Dev. Biol. Plant* 41:91–101.
- Rodrigues, H.C., L.M.C. Menezes, M. Cabral, G.M. Dornier, M. Rios, and M. Reynes. 2004. Evaluation of reverse osmosis and osmotic evaporation to concentrate camu-camu juice (*Myrciaria dubia*). *J. of Food Engin.* 63(1):97–102.
- Schuch, M.W. and A.C. Erig. 2005. Micropropagação de plantas frutíferas. In: *Propagação de plantas frutíferas*. 1ªed. Brasília: Embrapa Informações Tecnológicas. p. 155–173.
- Silva, M.A., P.J.A. Sobra, and T.G. Kieckbusch. 2006. State diagrams of freeze-dried camu-camu (*Myrciaria dubia* (HBK) Mc Vaugh) pulp with and without maltodextrin addition. *J. Food Engin.* 77(3):426–432.
- Souza, J.A., M.W. Schuch, L.C. Silva, J. Ferri, and G.C. Soares. 2007. Solidificante no meio de cultura e tamanho do explante no estabelecimento da propagação in vitro de pitangueira (*Eugenia uniflora* L.), *Revista Brasileira Agrociências, Pelotas.* 13(1):115–118.
- Teixeira, S.A., S.L. Chaves, and K. Yuyama. 2004. Esterases no exame da estrutura populacional de Camu-camu (*Myrciaria dubia* (Kunth) McVaugh-Myrtaceae). *Acta Amazônica.* 34(1):89–96.
- Yuyama, K., J.P.L. Aguiar, and L.K.O. Yuyama. 2002. Camu-camu: Um fruto fantástico como fonte de vitamina C. *Acta Amazonica, Manaus.* 32(1):169–174.
- Yuyama, K., N.B. Mendes, and J.P. Valente. 2011. Longevidade de sementes de camu-camu submetidas a diferentes ambientes e formas de conservação. *Revista Brasileira de Fruticultura.* 33(2):601–607.
- Yuyama, K.A. 2011. Cultura de camu-camu no Brasil. The culture of camu-camu in Brazil. *Revista Brasileira de Fruticultura.* 33(2): i–ii.
- Zanatta, C.F., E. Cuevas, F.O. Bobbio, P. Winterhalter, and A.Z. Mercadante. 2005. Determination of anthocyanins from camu-camu (*Myrciaria dubia*) by HPLC-PDA, HPLC-MS, and NMR. *J. Agric. and Food Chem.* 53(24):9531–9535.
- Zanatta, C. and A. Mercadante. 2007. Carotenoid composition from the Brazilian tropical fruit camu-camu (*Myrciaria dubia*). *Food Chem.* 101(4):1526–1532.