

In vitro germination and acclimatization of cambui tree type seedlings

Germinação *in vitro* e aclimatização de tipos de cambuzeiro

Ana da Silva Léo^{*} Luciana Borin Barin^{II} Ana Veruska Cruz da Silva^I
Francielen Paola de Sá^{III} Caroline de Araújo Machado^{IV}

ABSTRACT

There are few reports in literature on the *in vitro* behavior of cambui tree (*Myrciaria tenella* O. Berg) and acclimatization conditions. The aim of this study was to evaluate the effect of culture media on *in vitro* germination and the effect of different substrates on the acclimatization of two *Myrciaria tenella* types. The study was carried out at the Embrapa Tabuleiros Costeiros Laboratory of Plant Tissue Culture, Aracaju, SE. Seeds were extracted from fruits of two *Myrciaria tenella* types: Orange and Purple Types. The seeds were inoculated in the following culture media: T1 - MS medium + 30g L⁻¹ sucrose, T2 - ½ MS medium + 15g L⁻¹ sucrose and T3 - control without MS salts. To study the effect of substrates on acclimatization, seedlings were transferred to plastic containers with capacity of 300cm³ containing the following sterilized substrates: S1 - soil and powdered coconut husk - SPC (1:1 by volume); S2 - soil, washed sand and powdered coconut husk - SAPC (1:1:1 by volume) and S3 - Biomix[®] commercial substrate - SC. The medium without MS salts promoted 100% *in vitro* germination and ½ MS medium greater development of seedlings. All substrates studied are suitable for acclimatization of seedlings germinated *in vitro*. *Myrciaria tenella* of yellow type showed greater vigor during acclimatization.

Key words: *Myrciaria tenella* O. Berg, *in vitro* propagation, substrates, fruticulture.

RESUMO

São poucos os relatos na literatura sobre estudos do comportamento do cambuzeiro (*Myrciaria tenella* O. Berg) *in vitro* e condições de aclimação. O objetivo do trabalho foi de avaliar o efeito de meios de cultura na germinação *in vitro* e o efeito de diferentes substratos na aclimação de tipos de cambuzeiros. O estudo foi realizado no Laboratório de Cultura de Tecidos de Plantas da Embrapa Tabuleiros Costeiros, Aracaju,

SE. Foram utilizadas sementes extraídas de frutos de dois tipos de cambuzeiros: Tipo Laranja e Tipo Roxo. Para o estudo de meios para a germinação, as sementes foram inoculadas nos seguintes meios de cultura: T1 - meio de MS + 30g L⁻¹ de sacarose, T2 - meio ½ MS + 15g L⁻¹ de sacarose e T3 - testemunha sem sais MS. Para o estudo do efeito de substratos na aclimação, as plântulas foram transferidas para recipientes plásticos com capacidade de 300cm³, contendo os seguintes substratos esterilizados: S1 - solo e pó de casca de coco seco - SPC (1:1, em volume); S2 - solo, areia lavada e pó de casca de coco seco - SAPC (1:1:1, em volume); e S3 - substrato comercial Biomix[®] - SC. O meio sem sais MS proporciona 100% porcentagem de germinação *in vitro* e o meio ½ MS maior desenvolvimento em altura de plântulas. Todos os substratos estudados são adequados para aclimação de plântulas germinadas *in vitro*. O cambuzeiro do tipo Amarelo apresenta maior vigor na aclimação.

Palavras-chave: *Myrciaria tenella* O. Berg, propagação *in vitro*, substratos, fruticultura.

INTRODUCTION

Among the fruit species native to Brazil that are little known regarding sources of nutrients, the cambui tree (*Myrciaria tenella* O. Berg) stands out, belonging to the family *Myrtaceae*, which occurs from Maranhão down to Rio Grande do Sul, Brazil, extending up to Argentina. Some natural populations have already been observed in Sergipe, specifically in Itaporanga d'Ajuda and Pirambu regions (PINHEIRO et al., 2011). Its fruits are glabrous and bright globose berries, red or dark purplish when ripe. Besides the

^IEmbrapa Tabuleiros Costeiros, 490250-040, Aracaju, SE, Brasil. E-mail: ana.ledo@embrapa.br. *Autor para correspondência.

^{II}Departamento de Engenharia Florestal, Universidade Federal de Sergipe (UFS), São Cristóvão, SE, Brasil.

^{III}Programa de Pós-graduação em Biotecnologia de Recursos Naturais, UFS, São Cristóvão, SE, Brasil.

^{IV}Programa de Pós-graduação de Agricultura e Biodiversidade, UFS, São Cristóvão, SE, Brasil.

production of fruits, it has importance to landscaping and timber production (LORENZI, 2000), also being source of vitamin C (PINHEIRO et al., 2011) and having anti-inflammatory activity (APEL et al., 2010).

There are few reports in literature on the *in vitro* behavior of *Myrciaria tenella* and acclimatization conditions. The species is still in the domestication process and therefore all aspects related to its cultivation still need further studies. *In vitro* propagation technologies well developed and/or adapted for *Myrciaria tenella* are of great importance for conservation programs of genetic resources and genetic improvement of the species.

The establishment of protocols for the micropropagation of woody species from explants originating from plants grown *in vitro* is more feasible under the physiological and experimental point of view due to the juvenile stage and greater *in vitro* response, making the performance of several experiments possible (GRATTAPAGLIA & MACHADO, 1998). *In vitro* seed germination often allows greater seed germination than in nurseries, probably because *in vitro* conditions are more suitable for germination processes and early seedling development (NOLETO & SILVEIRA, 2004), such as observed for *Dendrocalamus membranaceus* Munro (BRAR et al., 2013) and *Cypripedium* (ZENG et al., 2013).

Several studies have been conducted with *in vitro* germination of native species such as *Hancornia speciosa* Gomes (MACHADO et al., 2004; LÉDO et al., 2007a), *Byrsonima intermedia* A. Juss. (NOGUEIRA et al., 2004), *Cereus jamacaru* DC. (RÊGO et al., 2009), *Genipa americana* L. (ALMEIDA et al., 2013) and exotic species *Moringa oleifera* Lam. (FREIRE et al., 2008) and *Azadirachta indica* A. Juss. (LÉDO et al., 2008).

Plant acclimatization has been a great obstacle in *in vitro* micropropagation of many species. Substrate, due to its chemical, physical and biological characteristics, has great influence on adaptation and early development of plants under natural conditions. It is therefore crucial to determine the suitable substrates for acclimatization, which should ensure mechanical support of the root system, plant stability, supply of water and nutrients and gas exchange between roots and atmospheric air. The use of alternative substrates, which are viable for acclimatization, is of great important, since the use of agro-industrial residues in agricultural practices has proven to be an alternative to solve social and environmental problems (SILVEIRA et al., 2002).

The use of powdered coconut husk in the composition of substrates has been promising

for the production of seedlings of different species (CORREIA et al., 2003; TERCEIRO NETO et al., 2004; BOMFIM et al., 2007; LÉDO et al., 2007b; SILVA et al., 2007). Besides high production, low cost and high availability, it is a suitable use for agro-industrial coconut residues (CARRIJO et al., 2002).

The aim of this study was to evaluate the effect of culture media on *in vitro* germination and the effect of different substrates on the acclimatization of two *Myrciaria tenella* types.

MATERIAL AND METHODS

The study was carried out at the Embrapa Tabuleiros Costeiros Laboratory of Plant Tissue Culture, Aracaju, SE. Seeds were extracted from fruits of two *Myrciaria tenella* types: Orange Type and Purple Type, derived from the natural population of Embrapa experimental field located in the municipality of Itaporanga d'Ajuda, Sergipe, Brazil (11°07'S and 37°10'W).

After extraction, seeds were submitted to disinfection with 70% alcohol for 1 minute, 2-2.5% commercial sodium hypochlorite for 20 minutes and triple washing in sterile water. The seeds were inoculated in the following culture media: T1 - MS medium + 30g L⁻¹ sucrose (MURASHIGE & SKOOG, 1962), T2 - ½MS medium + 15g L⁻¹ sucrose and T3 - control without MS salts (0MS). After inoculation, the flasks were kept in a growth chamber (25 ± 2 °C, 12 hours light and luminous intensity of 60 μmol m⁻²s⁻¹).

The experiment was completely randomized in a 2x3 factorial design (two *Myrciaria tenella* types x three culture media) with five replicates. Each experimental plot consisted of two test tubes with one seedling each. The germination percentage and the height of seedling at 120 days after inoculation were evaluated.

To study the effect of substrates on acclimatization, the seedlings were transferred to plastic containers with capacity of 300cm³ containing the following sterilized substrates: S1 -soil and powdered coconut husk - SPC (1:1 v/v); S2 - soil, washed sand and powdered coconut husk - SAPC (1:1:1 v/v/v) and S3 - Biomix[®] commercial substrate - SC. The experiment was completely randomized in a 2x3 factorial design (two *Myrciaria tenella* types x three culture media) with five replicates. Each experimental plot consisted of two test tubes with one seedling each.

The seedlings were acclimatized for 60 days in shading greenhouse at 50% with micro

sprinkler irrigation system. Supplementation of macro and micronutrients was held every seven days using a solution with half salt concentration of the MS medium. About 60 days after transfer to *ex vitro* conditions, the following evaluations were performed: survival percentage and number of leaf pairs.

The means of variables were submitted to analysis of variance and compared by the Tukey test at 5% significance level using the SISVAR software (FERREIRA, 2011).

RESULTS AND DISCUSSION

In vitro germination

There was a significant effect of the culture medium on the *in vitro* germination percentage. In the absence of MS salts, there was 100% of germination percentage (Table 1). Similar results were observed for *Hancornia speciosa* Gomes native of northeastern Brazil (LÉDO et al., 2007a), *Moringa oleifera* (FREIRE et al., 2008) and *Genipa americana* L. (ALMEIDA et al., 2013), these species presented higher *in vitro* germination in the absence of MS salts. Whereas the cotyledons have the function of storing reserve substances to become autotrophic seedling, so the presence of nutrient solution in the external environment becomes unnecessary for the *in vitro* germination of cambui tree.

Another aspect to be considered is that the presence of MS salts and sucrose may have contributed to reduce the osmotic potential of the medium, reducing the availability of water for inhibiting the seed germination. According RAJJOU

et al. (2012) the limited condition of presence of water allows sufficient hydration and increase of metabolic processes and repair preventing the germination.

No effect of *Myrciaria tenella* type on the germination percentage was observed. MACHADO et al. (2004) assessed the potential of *in vitro* propagation of 11 *Hancornia speciosa* Gomes matrixes native to the Brazilian Cerrado and observed 92.4% germination in MS medium without growth regulators and no differences between the matrixes were observed.

The height of seedlings showed significant effect only for culture medium (Table 1). Seedlings kept in culture medium ½ MS showed, on average, greater height development, with no differences between types and interaction between factors. The levels of organic and inorganic nutrients in *in vitro* culture media influenced various metabolic processes, showing effect on growth and tissues differentiation (MALDANER et al., 2006). Probably, reducing the concentration of salts in 50% had a positive effect on shoot growth.

Culture media without the presence of MS salts and half concentration of MS salts showed potential for the establishment of protocols for *in vitro* *Myrciaria tenella* propagation, lowering production costs due to its lower demand of salt concentration in the MS medium for the development of seedlings.

Acclimatization

There was no effect of *Myrciaria tenella* type, substrate or the interaction between factors on seedling survival in the acclimatization phase (Table 2). Although not statistically different, substrates added of powdered coconut husk (SPC and SAPC) showed numerical values greater than the commercial substrate. In studies conducted with coconut plants originated from zygotic embryo culture, LÉDO et al. (2007b) observed that the addition of powdered coconut husk to wash sand at a ratio of 1:1 (v/v) led to higher plant shoot growth and more leaves. *Saintpaulia ionantha* Wendl seedlings showed good growth during acclimatization in Plantagro® and Bioplant® commercial substrates, followed by powdered coconut husk and vermiculite (TERCEIRO NETO et al., 2004).

There was an effect of *Myrciaria tenella* type on the emission of new leaves in seedlings in the acclimatization phase (Table 2). There was no effect of substrate or interaction of factors on the number of leaf pairs.

The *Myrciaria tenella* seedlings yellow type showed, on average, highest growth in leaf area

Table 1 - *In vitro* germination percentage and height (cm) of *Myrciaria tenella* seeds in different culture media.

Culture medium	Orange type	Purple type	Means
	-----germination percentage-----		
MS + 30g L ⁻¹ sucrose	50.00	50.00	50.00B*
½ MS + 15g L ⁻¹ sucrose	50.00	50.00	50.00B
0 MS + 0g L ⁻¹ sucrose	100.00	100.00	100.00A
Means	83.33a	83.33a	
VC (%)	12.42		
	-----height (cm)-----		
MS + 30g L ⁻¹ sucrose	3.25	2.64	2.95B
½ MS + 15g L ⁻¹ sucrose	3.33	4.95	4.14A
0 MS + 0g L ⁻¹ sucrose	2.95	3.30	3.13AB
Means	3.17a	3.76a	
VC (%)	30.53		

*Means followed by the same capital letter in column and small letter in row do not differ from each other at 5% by the Tukey test.

Table 2 - Survival rate and number of leaf pairs emitted of *Myrciaria tenella* seedlings acclimated to different substrates.

Substrate	Orange type	Purple type	Means
-----Survival rate-----			
SPC*	91.67	83.33	87.50A**
SAPC	91.67	100.00	95.83A
SC	90.91	69.23	79.17A
Means	91.43a	83.78a	
VC (%)	33.46		
-----Number of leaf pairs-----			
Substrate			Means
SPC	3.58	2.33	2.95A
SAPC	2.91	2.50	2.70A
SC	3.00	2.15	2.54A
Means	3.17a	2.32b	
VC (%)	23.93		

* S1 - soil and powdered coconut husk - SPC (1:1 v/v); S2 - soil, washed sand and powdered coconut husk - SAPC (1:1:1 v/v/v) and S3 - Biomix® commercial substrate - SC.

**Means followed by the same capital letter in column and small letter in row do not differ from each other at 5% by the Tukey test.

in the acclimatization phase with the emission of 3.17 leaf pairs. According SAEIDI (2008), the genetic variation and de vigor seed indicates the attempts to the genetically improve these seed quality traits that should be successful and optimistic in breeding programs. In genetic diversity studies of natural populations of *Myrciaria tenella* of yellow, orange and purple types, PINHEIRO et al. (2011) found a high genetic diversity among types. Probably, the yellow type showed a greater strength under acclimatization conditions of shading greenhouse.

CONCLUSION

For the *in vitro* sexual propagation and seedlings development of the *Myrciaria tenella* it is recommend the culture medium without MS salts and ½ MS with sucrose 15g L⁻¹. All the substrates tested SPC (1:1 v/v); SAPC (1:1:1 v/v/v) and Biomix® are appropriated for seedlings acclimatization. The *Myrciaria tenella* yellow type showed highest growth in leaf area during acclimatization.

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