CROP BREEDING AND APPLIED BIOTECHNOLOGY

ARTICLE

Large-scale, high-efficiency production of coffee somatic embryos

Anna Lygia de Rezende Maciel[1], Filipe Almendagna Rodrigues[2*], Moacir Pasqual[2] and Carlos Henrique Siqueira de Carvalho[3]

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Abstract: *The study aims to compare the efficiency of previously used liquid media for coffee, evaluating NAA concentrations in liquid medium and proline in semi-solid medium in the regeneration of somatic embryos and asses concentrations of BA and IAA in the maturation of embryos in temporary immersion bioreactors. For the regeneration of globular embryos from embryogenic aggregates and calli, we tested five concentrations (0.00, 0.25, 0.50, 1.00 and 2.00 mg L^{-1}) of NAA in liquid medium and five concentrations (0.0, 0.5, 1.0, 2.0 and 4.0 g L^{-1}) of proline in semisolid medium. The multiplication of embryogenic aggregates was highest in culture medium MM, reaching a density 7.5 times greater than that of the initial density. NAA promoted a linear increase in embryo regeneration. The medium containing 2.0 mg L^{-1} BA and 0.0 mg L^{-1} IAA yielded the highest percentage of large cotyledonary embryos.*

Key words: *Vegetative propagation, biotechnology, tissue culture,* Coffea arabica L.

INTRODUCTION

The vegetative propagation of coffee (*Coffea arabica* L.) via somatic embryogenesis facilitates the rapid evaluation of F1 hybrids and segregating genotypes in genetic breeding programs, allowing clonal varieties to be developed in only 10 years (Caixeta et al. 2008, Salgado et al. 2014). Large-scale multiplication via somatic embryogenesis has been used for the dissemination of F1 hybrids with high levels of heterosis in Central America (Bertrand et al. 2011) and is becoming a reality in Mexico (Etienne et al. 2013) and Brazil (Carvalho et al. 2013), in part due to advances in the use of temporary immersion bioreactors for the production of pregerminated embryos (Ducos et al. 2007b). While the cost-effectiveness of somatic embryogenesis for commercial coffee propagation remains unsatisfactory, profitability could be enhanced by optimizing the multiplication protocol thus decreasing the production cost per somatic seedling (Etienne et al. 2013). The concentrations and relative amounts of auxin and cytokinin vary greatly among protocols for the in vitro cultivation of coffee. For embryogenic callus multiplication, Teixeira et al. (2004) suggested the use of 1.0 mg L^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), 1.0 mg L^{-1} indolebutyric acid (IBA), and 2.0 mg L^{-1} 2-isopentenyl adenine (2-ip), while Menéndez-Yuffá et al. (2010) and Etienne et al. (2005) used 1.0 mg L^{-1} 2,4-D and 1.0 mg L^{-1} kinetin.

The regeneration of somatic embryos in coffee has been achieved with a

***Corresponding author:**
E-mail: filipealmendagna@yahoo.com.br

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[1] Instituto Federal de Educação, Ciência e Tecnologia do Sul de Minas Gerais, Campus Morro Preto, 37.890-000, Muzambinho, MG, Brazil
[2] Universidade Federal de Lavras, Departamento de Agricultura, Campus da UFLA, 3037, 37.200-000, Lavras, MG, Brazil
[3] Embrapa Café, Alameda do Café, 1000, Jardim Andere, 37.026-400, Varginha, MG, Brazil

wide range of growth regulators. Bertrand et al. (2011), Etienne et al. (2005), and Menéndez-Yuffá et al. (2010) reported the use of a combination of adenine sulfate and 6-benzylaminopurine (BA), while Afreen et al. (2002) used IBA, adenine, and BA and Papanastasiou et al. (2008) and Albarrán et al. (2005) used only BA. Teixeira et al. (2004) reported that 0.25 mg L^{-1} naphthaleneacetic acid (NAA) can efficiently regenerate globular embryos in semisolid medium, but that regeneration in liquid medium is variable.

After induction of embryogenesis, which is usually conducted in Erlenmeyer flasks, Petri dishes (Carvalho et al. 2013), or bioreactors (Barry-Etienne et al. 2002), the globular stage embryos are transferred to temporary immersion bioreactors for growth and maturation. BA is commonly added at this phase (Pereira et al. 2007, Etienne et al. 2013) to assist embryo development. However, it is desirable that the cotyledonary embryos develop a root system to support plant growth during acclimatization (Ducos et al. 2007b). Therefore, a combination of cytokinin and auxin has been suggested for application during embryo maturation (Andrade et al. 2001, Teixeira et al. 2004).

The study aims to compare the efficiency of previously used liquid media for coffee, evaluating NAA concentrations in liquid medium and proline in semi-solid medium in the regeneration of somatic embryos and asses concentrations of BA and IAA in the maturation of embryos in temporary immersion bioreactors.

MATERIAL AND METHODS

Four experiments were conducted with three mother plants of Siriema, a *Coffea arabica* L. population derived from a cross between *Coffea racemosa* x *Coffea arabica* with resistance to leaf rust and leaf miners.

The culture media used in this study are described in Table 1. The pH of all culture media was adjusted to 5.6 ± 0.1 and addition of 2.8 g L^{-1} Phytagel® (Sigma, St. Louis, USA). The media were autoclaved at 121 °C and 1 atm for 20 minutes. Only gibberellic acid (GA_3) and 2-isopentenyl adenine (2-ip) were filtered and added to the media after autoclaving.

Embryogenic calli were induced from young, fully expanded leaves using PM and SM media, according to the protocol described by Teixeira et al. (2004) but modified to increase the concentrations of 2,4-D and 2-ip in the PM medium (here called MI medium) to 4.42 mg L^{-1} and 4.1 mg L^{-1}, respectively. The embryogenic calli were cultured in Petri dishes for three months before the experiments.

In a laminar flow hood, 20 mL of T3 or MM liquid media were added to 125-mL Erlenmeyer flasks and inoculated with 200 mg of embryogenic callus. The flasks were sealed with aluminum foil and placed in an orbital shaker at 100

Table 1. Culture media used during experiments with *Coffea arabica* L.

Components	Concentration (mg L^{-1})					
	MI[1]	T3[2]	SM[3]	MM[3]	RM[3]	RR[4]
MS salts (Murashige and Skoog 1962)	½-MS[5]	½-MS	½-MS	½-MS	½-MS	½-MS
Thiamine HCl	10.0	5.0	10.0	10.0	10.0	10.0
Pyridoxine HCl	1.0	0.5	1.0	1.0	1.0	1.0
Nicotinic acid	1.0	0.5	1.0	1.0	1.0	1.0
Glycine	1.0	-	1.0	1.0	1.0	1.0
Myoinositol	100.0	100.0	100.0	100.0	100.0	100.0
Casein hydrolysate	100.0	100.0	100.0	100.0	100.0	-
Malt extract	400.0	200.0	400.0	200.0	400.0	-
Citric acid	-	-		250.0	-	-
Kinetin	-	1.0		-	-	-
2,4-D	4.42	1.0	2.21	1.0	-	-
IBA		-		1.0	-	-
NAA	-	-	-	-	0.25	20.0
2-ip	4.1	-	2.0	2.0	-	
BA	-	-	-	-	2.0	2.0
Sucrose (g)	20.0	30.0	20.0	20.0	30.0	30

[1] PM medium (Teixeira et al. 2004) with 4.4 mg L^{-1} 2,4-D and 4.1 mg L^{-1} 2-ip. [2] Boxtel and Berthouly (1996). [3] Teixeira et al. (2004). [4] RM medium (Teixeira et al. 2004) without malt extract and casein hydrolysate and with 20.0 mg L^{-1} NAA. [5] ½-strength Murashige and Skoog salts (Murashige and Skoog 1962).

rpm, in the dark, at 26 °C ± 2 °C (Etienne et al. 2005). After one month, the suspensions of the embryogenic aggregates had been separated by color (yellow or whitish) and were used to start the experiment. The cultures were subcultured every 15 days.

A completely randomized 2 x 2 factorial design with six repetitions was used to compare the multiplication rates of embryogenic aggregates with different colors (yellow or whitish) (Ribas et al. 2011) in the two liquid media T3 (Boxtel and Berthouly 1996) and MM (Teixeira et al. 2004) (Table 1).

The final mass (FM) in g Erlenmeyer flask^{-1}; the number of mass increases, defined as (FM/initial mass); the final density (FD) in g L^{-1}; and the percentage of mass increase for the embryonic aggregates, (I%) calculated by the formula [(FM*100/ Initial mass) – 100] were evaluated after 60 days.

The effect of NAA on the regeneration of globular embryos was evaluated using embryogenic aggregates previously grown in T3 liquid medium. The treatments consisted of five different concentrations of NAA (0.00, 0.25, 0.50, 1.00 and 2.00 mg L^{-1}) added to RM culture medium (Teixeira et al. 2004) (Table 1) without malt extract and casein hydrolysate and supplemented with 1.0 g L^{-1} proline. A completely randomized design with six repetitions was used. Each repetition consisted of 20 mL of liquid medium in a 125-mL Erlenmeyer flask inoculated with 20 mg of yellow embryogenic aggregate. The flasks were sealed with aluminum foil, placed in an orbital shaker at 100 rpm in the dark at 26 °C ± 2 °C, and subcultured every 15 days (Teixeira et al. 2004). The total number of regenerated globular embryos was determined 60 days after the start of the experiment.

To study the effect of proline on the regeneration of somatic embryos, a modified RM medium (Teixeira et al. 2004) designated as RR medium was used. RR consists of RM medium without malt extract and casein hydrolysate and with 20 mg L^{-1} NAA. Proline at concentrations of 0.0, 0.5, 1.0, 2.0 and 4.0 g L^{-1} was added to the RR medium. Four 100 mg sectors of embryogenic aggregate grown in T3 liquid medium for two months were placed in 90 mm diameter Petri dishes and kept in the dark at 26 °C ± 2 °C. The calli were subcultured every 30 days for 120 days. After this period, the total number of globular embryos per sector was estimated. A completely randomized design with five Petri dishes per treatment was used.

Globular embryos regenerated in Petri dishes with the RR medium (Table 2) were used to determine the effects of different combinations of BA and indole-3-acetic acid (IAA) on the regeneration of globular embryos. The following combinations of BA and IAA were tested in the RM medium (Teixeira et al. 2004) (Table 1): 2.0/0.0, 0.25/0.0, 0.25/0.25, 0.25/0.50, and 0.50/0.50 BA/IAA mg L^{-1}.

The statistical design for this experiment was completely randomized with six repetitions, i.e., six 1-L RITA temporary immersion type bioreactors (CIRAD, Montpellier, France). Each RITA was inoculated with approximately 500 globular embryos and 200 ml of culture medium, then placed in the dark at 26 °C ± 2 °C. Through an automated pumping system, the embryos were immersed in the medium for two minutes every 12 hours.

After six weeks of subculturing, each medium was renewed and supplemented with 0.5 mg L^{-1} gibberellic acid (GA_3). The embryos remained in the dark for four additional weeks and were then transferred to a light room with a 12 h photoperiod and light intensity of 50 µmol m^{-2} s^{-1} (Etienne 2005).

Table 2. Number of cotyledonary embryos; percentages of small (0.50-1.5 cm), medium (1.6-2.5 cm), and large (2.6-5.0 cm) cotyledonary embryos; and percentages of small, medium, and large cotyledonary embryos with roots per RITA grown with different concentrations of BA and IAA in *Coffea arabica* L.

Growth regulators (mg L^{-1})		Number of embryos	Embryo size (%)			Embryos with roots (%)		
BA	IAA		Small	Medium	Large	Small	Medium	Large
2.00	0.00	522.8a	15.8c	49.0a	34.3a	45.5a	90.3a	90.8a
0.25	0.00	511.3a	53.4a	31.0b	17.2b	35.3a	90.2a	79.5a
0.25	0.25	506.8a	44.7a	35.7b	19.8b	43.7a	91.2a	100.0a
0.25	0.50	496.8a	34.5b	43.8ª	20.0b	53.7a	92.8a	98.8a
0.50	0.50	523.8a	34.3b	48.2ª	17.0b	60.2a	85.5a	100.0a
Mean		512.3	36.5	41.5	21.7	47.7	90.0	93.8

Means followed by the same letter in the same column do not differ significantly according to the Scott-Knott test at 5% probability.

Table 3. Final mass, number of mass increases, final density, and percentage of mass increase of embryogenic aggregates per Erlenmeyer flask after growth in the liquid media T3 and MM for 60 days

Culture media	Embryogenic aggregates Erlenmeyer flask^{-1}			
	Mass (g)	No. of mass increases	Final density (g L^{-1})	Mass increase (%)
T3	0.98b	4.92b	39.02b	392.3b
MM	1.69a	8.46a	74.54a	746.1a

Means followed by the same letter in the same column do not differ significantly by the Scott-Knott test at 5% probability. T3 (Boxtel and Berthouly 1996). MM (Teixeira et al. 2004).

After 84 days under these light conditions, the total number of cotyledonary embryos per bioreactor, the percentage of small (0.50-1.5 cm), medium (1.6-2.5 cm) and large (2.6-5.0 cm) cotyledonary embryos per RITA, and the percentage of embryos with roots in each size category were evaluated.

The data were submitted to statistical analysis with the F test at 5% probability, and the means were compared with the Scott-Knott cluster test and polynomial regression using the SISVAR statistical software program (Ferreira 2011).

RESULTS AND DISCUSSION

The embryogenic aggregates showed higher multiplication in the culture medium MM (Table 3). The color of the embryogenic aggregates did not affect the rate of multiplication. The number of increases of the embryogenic aggregates was higher in the MM liquid medium. It should be noted that the concentration of 2,4-D in the MM medium was not appreciably higher than that in the T3 medium, 0.50 and 0.45 mg L^{-1}, respectively, because high concentrations of 2,4-D have been associated with somaclonal variation in coffee plants regenerated from tissue cultures (Duncan 1998), which is undesirable for commercial propagation.

The size of the cotyledonary embryo is likely affected by the number of embryos produced per RITA. Etienne et al. (2006) reported an average embryo size of 1.0-1.2 cm for a RITA with 800 cotyledonary embryos, a much smaller size than found for the RITAs in the present study with 500 embryos each.

The final density of the embryogenic aggregates was higher in the culture medium MM than in T3, reaching a density 7.5 times greater than the initial density; this result was similar to that found by Teixeira et al. (2004) using the cultivar 'Catuaí Vermelho IAC 144.' As the Siriema population and 'Catuaí Vermelho IAC 144' have different genetic backgrounds (Carvalho et al. 2008), the MM medium can likely be used as the primary option for propagating various *Coffea arabica* clones in breeding programs.

The NAA concentrations followed a linear model. The highest concentration tested, 2.0 mg L^{-1} NAA, promoted the formation of 2.4 globular embryos per 1 mg of embryogenic aggregate (Figure 1), a result similar to that reported by Carvalho et al. (2013) for the regeneration of embryos in Petri dishes using 2.0 mg L^{-1} NAA, the level commonly indicated for the production of clonal plants in commercial laboratories. Teixeira et al. (2004), working

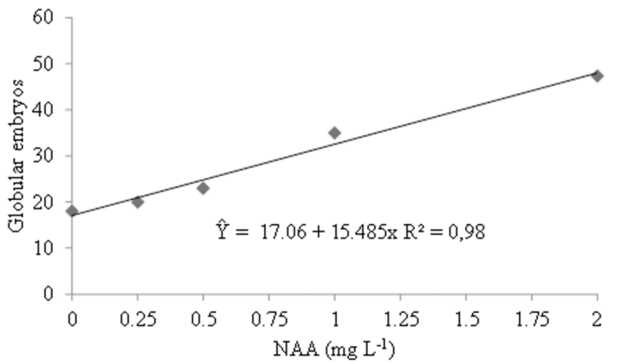

Figure 1. Number of globular embryos per 20 mg of embryogenic aggregates obtained through regeneration in RM liquid medium with different concentrations of naphthaleneacetic acid (NAA) in *Coffea arabica* L.

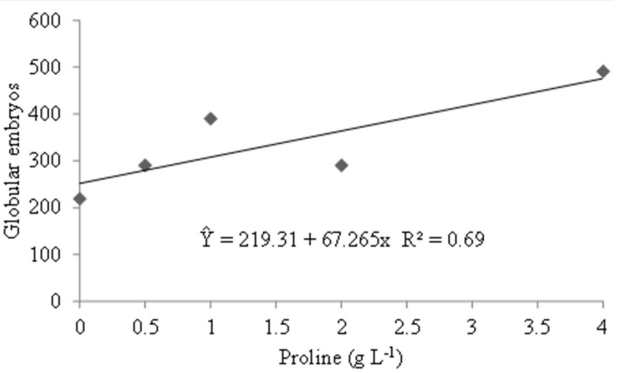

Figure 2. Number of globular embryos per 100 mg of callus obtained in RM medium with different concentrations of proline in *Coffea arabica* L.

with several coffee genotypes and using the RM medium with 0.25 mg L^{-1} NAA, found that 71.3% of the genotypes studied exhibited some degree of regeneration after two months of cultivation, suggesting that the use of NAA for regeneration is broadly applicable.

The number of globular embryos showed a linear increase as the concentration of proline increased (Figure 2). Proline at 4.0 mg L^{-1} produced 4 globular embryos per 1 mg of callus. The globular embryos that regenerated under this concentration were also larger than those that regenerated without proline (data not shown).

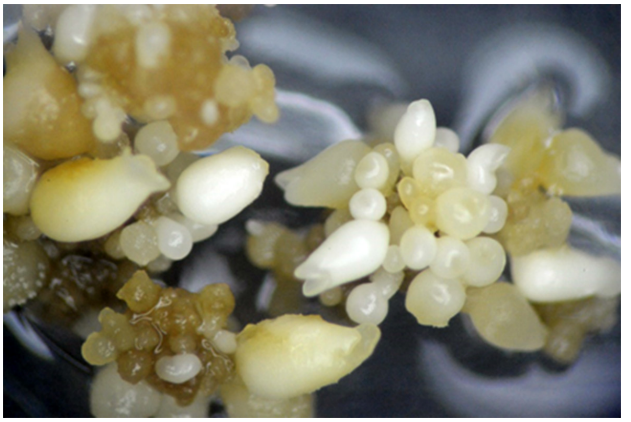

Figure 3. Formation of cotyledonary embryos of *Coffea arabica* L.

The studied concentrations of BA and IAA did not affect the conversion of globular embryos to cotyledonary embryos (Table 2), and all media tested resulted in 100% formation of cotyledonary embryos (Figure 3). However, the sizes of these cotyledonary embryos differed in the different culture media (Table 2). All treatments produced embryos in each of the three size categories: small, medium, and large. The production of cotyledonary embryos of different sizes is commonly observed in cylindrical bioreactors and may be due to the decreased light in the interior of the embryo mass inside the bioreactor (Ducos et al. 2007a). Barry-Etienne et al. (2002) obtained 86% germination of somatic embryos using RITA bioreactors, but these embryos exhibited heterogeneous anatomical features. Similarly, for *Coffea canephora,* Ducos et al. (2007b) reported heterogeneity in cotyledonary embryos grown in large (8 L) cylindrical temporary immersion bioreactors. The use of horizontal bioreactors reduces the heterogeneity of embryo size by exposing the embryo mass to more incident light (Ducos et al. 2007a).

The 0.25 mg L^{-1} BA/0.0 mg L^{-1} IAA and 0.25 mg L^{-1} BA/0.25 mg L^{-1} IAA treatments resulted in the greatest percentage of small cotyledonary embryos.

The medium with 2.0 mg L^{-1} BA/0.0 mg L^{-1} IAA produced the highest percentage of large cotyledonary embryos (2.6-5.0 cm). The decrease of the BA concentration from 2.0 mg L^{-1} to 0.25 mg L^{-1} increased the percentage of small, indicating the importance of this factor in the size of cotyledonary embryos. No treatment significantly affected the emergence of roots in the cotyledonary embryos (Table 2), but the percentage of small embryos with roots was lower than that of medium and large embryos.

In the present study, we improved several steps in the protocol for the large-scale production of somatic embryos. We found that the best conditions for coffee were as follows: multiplication of embryogenic aggregates with MM medium; regeneration of somatic embryos in liquid medium containing 2.0 mg L^{-1} NAA or in Petri dishes containing 4.0 mg L^{-1} proline; and the use of 2.0 mg L^{-1} BA for the production of cotyledonary embryos in RITA bioreactors. The results reported here have enabled the multiplication of clonal plants from different genetic backgrounds in the breeding program of the Fundação Procafé.

CONCLUSIONS

The culture medium MM provides a higher multiplication rate of embryogenic aggregates than does the medium T3. Increasing the concentration of NAA from 0.0 to 2.0 mg L^{-1} promotes a corresponding linear increase in the regeneration of *Coffea arabica* somatic embryos in liquid medium. The regeneration of *Coffea arabica* globular embryos in semisolid medium is directly proportional to the concentration of proline up to 4.0 g L^{-1}. The studied concentrations of BA and IAA did not affect the conversion of globular embryos to cotyledonary embryos, and all tested media resulted in 100% conversion and 2.0 mg L^{-1} BA/0.0 mg L^{-1} IAA combination produced the highest percentage of large cotyledonary embryos.

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REFERENCES

Afreen F, Zobayed SMA and Kozai T (2002) Photoautotrophic culture of *Coffea arabusta* somatic embryos: photosynthetic ability and growth of different stage embryos. **Annals of Botany 90**: 11-19.

Albarrán J, Bertrand B, Lartaud M and Etienne H (2005) Cycle characteristics in a temporary immersion bioreactor affect regeneration, morphology, water and mineral status of coffee (*Coffea arabica*) somatic embryos. **Plant Cell, Tissue and Organ Culture 81**: 27-36.

Andrade LMCO, Pasqual M, Maciel ALR, Pereira AB and Cavalcante-Alves JM (2001) Cultura *in vitro* de embriões de *Coffea arabica*: influência de NAA e BAP. **Ciência e Agrotecnologia 25**: 1063-1070.

Barry-Etienne D, Bertrand B, Sholonvoigt AN and Etienne H (2002) The morphological variability within a population of coffee somatic embryos produced in a bioreactor affects the regeneration and the development of plants in the nursery. **Plant Cell, Tissue and Organ Culture 68**: 153-162.

Bertrand B, Alpizar E, Lara L, Santacreo R, Hidalgo M, Quijano JM, Montagnon C, Georget F and Etienne H (2011) Performance of *Coffea arabica* F1 hybrids in agroforestry and full-sun cropping systems in comparison with American pure line cultivars. **Euphytica 181**: 147-158.

Boxtel J and Berthouly M (1996) High frequency somatic embryogenesis from coffee leaves. **Plant Cell, Tissue and Organ Culture 44**: 4-17.

Caixeta ET, Carvalho CHS, Zambolim EM, Pereira LFP and Sakiyama NS (2008) Biotecnologia aplicada ao desenvolvimento de cultivares de café. In Carvalho CHS (ed) **Cultivares de café: origem, características e recomendações**. Editora Embrapa, Brasília, p. 103-128.

Carvalho CHS, Fazuoli LC, Carvalho GR, Guerreiro Filho O, Pereira AA, Almeida SR, Matiello JB, Bartholo GF, Sera T, Moura WM, Mendes ANG, Rezende JC, Fonseca AFA, Ferrão MAG, Ferrão RG, Nacif AP, Silvarolla MB and Braghini MT (2008) Cultivares de café arábica de porte baixo. In Carvalho CHS (ed) **Cultivares de café: origem, características e recomendações**. Editora Embrapa, Brasília, p. 155-252.

Carvalho CHS, Paiva ACRS, Silva EQ and Custódio AA (2013) **Custo de produção de mudas clonais de café arábica produzidas por embriogênese somática**. Editora Embrapa, Brasília, 10p.

Duncan RR (1998) Tissue culture-induced variation and crop improvement. **Advances in Agronomy 58**: 201-240.

Ducos JP, Lambot C and Pétiard V (2007a) Bioreactors for coffee propagation by somatic embryogenesis. **The International Journal of Developmental Biology 1**: 1-12.

Ducos JP, Labbe G, Lambot C and Pétiard V (2007b) Pilot scale process for the production of pre-germinated somatic embryos of selected robusta (*Coffea canephora*) clones. **In Vitro Cellular & Developmental Biology - Plant 43**: 652-659.

Etienne H (2005) Somatic embryogenesis protocol: coffee (*Coffea arabica* L. and *C. canephora* P.). In Jain SM and Gupta PK (eds) **Protocol for Somatic Embryogenesis in Wood Plants**. Springer, Dordrecht, p. 167-179.

Etienne H, Bertrand B, Georget F, Lartaud M, Montes F, Dechamp E, Verdeil J and Barry-Etienne D (2013) Development of coffee somatic and zygotic embryos to plants differs in the developmental, histochemical and hydration aspects. **Tree Physiology 33**: 640-653.

Etienne H, Dechamp E, Barry-Etienne D and Bertrand B (2006) Bioreactors in coffee micropropagation. **Brazilian Journal of Plant Physiology 18**: 45-54.

Ferreira DF (2011) Sisvar: a computer statistical analysis system. **Ciência e Agrotecnologia 35**: 1039-1042.

Menéndez-Yuffá A, Barry-Etienne D, Bertrand B, Georget F and Etienne H (2010) A comparative analysis of the development and quality of nursery plants derived from somatic embryogenesis and from seedlings for large-scale propagation of coffee (*Coffea arabica* L.). **Plant Cell, Tissue and Organ Culture 102**: 297-307.

Papanastasiou I, Soukouli K, Moschopoulou G, Kahia J and Kintzios S (2008) Effect of liquid pulses with 6-benzyladenine on the induction of somatic embryogenesis from coffee (*Coffea arabica* L.) callus cultures. **Plant Cell, Tissue and Organ Culture 92**: 215-225.

Pereira AR, Carvalho SP, Pasqual M and Santos FC (2007) Embriogênese somática direta em explantes foliares de *Coffea arabica* cv. Acaiá Cerrado: efeito de cinetina e ácido giberélico. **Ciência e Agrotecnologia 31**: 332-336.

Ribas AF, Dechamp E, Champion A, Bertrand B, Combes M, Verdeil J, Lapeyre F, Lashermes P and Etienne H (2011) Agrobacterium-mediated genetic transformation of *Coffea arabica* (L.) is greatly enhanced by using established embryogenic callus cultures. **BMC Plant Biology 11**: 1-15.

Salgado SML, Rezende JC and Nunes JAR (2014) Selection of coffee progenies for resistance to nematode *Meloidogyne paranaensis* in infested area. **Crop Breeding and applied Biotechnology 14**: 94-101.

Teixeira JB, Junqueira CS, Pereira AJPC, Mello RIS, Silva APD and Mundim DA (2004) **Multiplicação clonal de café (*Coffea arabica* L.) via embriogênese somática**. Editora Embrapa, Brasília, 39p.