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Technical Note

DIRECT SOMATIC EMBRYOGENESIS IN *COFFEA ARABICA* L. CVS. CATURRA AND CATUAÍ: EFFECT OF TRIACONTANOL, LIGHT CONDITION, AND MEDIUM CONSISTENCY

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RESUMEN

Embriogénesis somática directa en *Coffea arábiga* L. cvs. Caturra y Catuaí: efecto del triacontanol, la condición de luz, y la consistencia del medio. Se investigó la influencia de la concentración de triacontanol (TRIA) y su interacción con el ácido indolacético (AIA) en la inducción de la embriogénesis somática directa en *Coffea arabica* L. cvs. Caturra y Catuaí. Adicionalmente, se evaluó el efecto de la condición de la luz y de la consistencia del medio de cultivo (semisólido vs. líquido). Se determinó que la mayor cantidad de embriones somáticos por explante fue de 3,9±0,5 en Caturra y 3,6±0,5 en Catuaí, en el medio de cultivo Murashige y Skoog (1962) al 50%, complementado con BAP (1,1 μM), IAA (2,85 μM) y TRIA (4,55 μM). Para la consistencia del medio, la mayor cantidad de embriones somáticos en Caturra (3,2±0,2) y Catuaí (6,0±0,4) se obtuvo con el medio semisólido de Yasuda *et al*. (1985). En Catuaí, la mayor cantidad de embrioides se obtuvo con 16 h luz (7,6±1,0) y oscuridad (6,2±0,6) y para Caturra, con oscuridad (4,2±0,4) y 16 h luz (3,8±0,5). No se observó la formación de embriones somáticos en los explantes de Caturra y Catuaí después de 12 semanas de cultivo, en el medio líquido descrito por Yasuda *et al*. (1985) ni bajo ninguna de las condiciones de luz.

ABSTRACT

The influence of triacontanol (TRIA) concentration and its interaction with indole-3-acetic acid (IAA) on direct somatic embryogenesis (DSE) of *Coffea arabica* L. cvs Caturra and Catuaí was determined. Additionally, light conditions and culture medium consistency (semisolid vs. liquid) were evaluated. A higher average of somatic embryos per explant from Caturra (3.9±0.5) and Catuaí (3.6±0.5) leaves was obtained when 4.55 μM TRIA was added to the half-strength Murashige and Skoog (1962) medium supplemented with 1.1 μM benzilaminopurine (BAP) and 2.85 μM IAA. In relation to medium consistency, the highest number of embryoids in Caturra (3.2±0.2) and Catuaí (6.0±0.4) explants was obtained using Yasuda *et al*. (1985) semisolid medium. Regarding photoperiod, the number of embryoids per explant obtained for Catuaí cultured under a 16 h light photoperiod was 7.6±1.0 and in the dark 6.2±0.6. In Caturra, 4.2±0.4 embryoids were produced in the dark and 3.8±0.5 with 16 h light. No somatic embryos were observed in Caturra and Catuaí explants after 12 weeks of culture in liquid Yasuda *et al*. (1985) medium, and under any of the light conditions.

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INTRODUCTION

Improvement of coffee varieties through genetic engineering requires an efficient *in vitro* culture system for obtaining competent explants that could be used as target tissue for transformation and plant regeneration (Fernández-Da Silva and Menéndez-Yuffá 2003). In this sense somatic embryogenesis is the most suitable method, it consists in a developmental process by which somatic cells develop into somatic embryos through an orderly series of characteristic embryological stages without fusion of gametes (Jiménez 2005). For coffee this process can be achieved via direct somatic embryogenesis (DSE), indirect somatic embryogenesis (ISE) or secondary somatic embryogenesis (SSE).

DSE is characterized by the induction of somatic embryos directly from pro-embryogenic cells from leaves, stem, microspores or protoplasts without the proliferation of calli, whereas in ISE somatic embryos are developed from friable embryogenic calli (Jiménez 2001, Molina *et al*. 2002). SSE is the process by which somatic embryos are formed from primary embryos (Raemakers *et al*. 1995).

DSE presents some advantages over ISE, such as the shorter period needed for obtaining somatic embryos (Yasuda *et al*. 1985, Hatanaka *et al*. 1991, Bieysse *et al*. 1993, Van Boxtel and Berthouly 1996, Loyola-Vargas *et al*. 1999, Giridhar *et al*. 2004, Gatica *et al*. 2007), the reduction on the culture time that might decrease the frequency of somaclonal variation (Etienne and Bertrand 2001, Barry-Etienne *et al*. 2002ab, Etienne and Bertrand 2003), and it could be the only procedure for obtaining somatic embryogenesis for those coffee genotypes that are recalcitrant to ISE (Van Boxtel and Berthouly 1996).

The DSE process has been described for coffee by Yasuda *et al*. (1985), Hatanaka *et al*. (1991), Bieysse *et al*. (1993), Fuentes *et al*. (2000), Fuentes-Cerda *et al*. (2001), Molina *et al*. (2002), Giridhar *et al*. (2004), and Gatica *et al*. (2007). These investigations have focused on studying the effect of nutrients, the plant growth regulators, the genotype, the source and the explant age on DSE. Nevertheless, the influence of TRIA concentration, light conditions and medium consistency on DSE induction from explants of Caturra and Catuaí coffee varieties has not been reported.

TRIA, a 30-carbon primary alcohol, is a naturally occurring plant growth promoter and it is known to stimulate morphogenetic responses under *in vitro* conditions (Reddy *et al*. 2002, Giridhar *et al*. 2004). The effect of TRIA on shoot multiplication and rooting was tested on *Capsicum frutescens* and *Decalepis hamiltonii* (Reddy *et al*. 2002), *Thymus mastichina* (Fraternale *et al*. 2003), *Malus domestica* cv. JTE-E4 and *Cerasus fruticosa* cv. Probocskai (Tantos *et al*. 2001), and *Costus speciosus* (Malabadi *et al*. 2005). Giridhar *et al*. (2004), also showed that TRIA enhances somatic embryogenesis induction in *C. canephora* cv. 274 and *C. arabica* cv. Hemavathy.

The condition and intensity of light has been reported to affect somatic embryogenesis in terms of induction and growth of somatic embryos on *Glycine max* (Lazzeri *et al*. 1987), *Araujia sericifera* (Torné *et al*. 2001), and *Cydonia oblonga* (Morini *et al*. 2000).

The aim of this work was to determine the influence of TRIA concentration, light conditions and culture medium consistency (semisolid vs. liquid) on DSE induction in *Coffea arabica* L. cvs. Caturra and Catuaí.

MATERIALS AND METHODS

Plant material

Sections from vitroplants leaves of Caturra and Catuaí (*Coffea arabica* L.) were used as explants to induce DSE. Seeds of Caturra and Catuaí, collected from coffee plantations in Alajuela, Costa Rica, were washed and soaked for 24 h in distilled water with 2 drops of Tween 20, disinfected with 3.5 % (v/v) sodium hypochlorite for 1 h and finally rinsed 3 times with sterile distilled water. The zygotic embryos were excised from the seeds and cultured in baby food jars, closed with polyethylene food wrap (Glad,

Costa Rica), containing 20 ml of MS medium (Murashige and Skoog, 1962) supplemented with Morel vitamins (Morel 1965), 100 mg.l^{-1} myo-inositol, 200 mg.l^{-1} casein hydrolysate, 400 mg.l^{-1} malt extract, 4.4 µM BAP and 2 g.l^{-1} Gelrite. The pH was adjusted to 5.6 before autoclaving for 21 min at 121 °C and 1.07 kg cm^{-2}. In vitro plantlets developed from these embryos were cultured with 20 ml of the above medium under a 16 h light photoperiod (30 μmol.m^{-2}.s^{-1}) at 26±2°C and transferred to fresh medium every 90 days.

Influence of culture medium consistency and light conditions on DSE

In a first experiment, the interaction between culture medium consistency and light conditions on DSE was evaluated. Leaf explants of 0.5 cm^2 from the first and second pair of leaves of vitroplants, without the midvein and the margins, were cultured on semisolid and liquid Yasuda *et al.* (1985) culture medium. The explants were cultured under three different light conditions: continuous white light (30 μmol.m^{-2}.s^{-1}), 16 h light photoperiod (30 μmol.m^{-2}.s^{-1}) or in the dark. Semisolid media were gelled with 2 g.l^{-1} Gelrite. The explants were cultured in Petri dishes and in 125 ml Erlenmeyer flasks containing 10 ml of liquid medium and maintained on a shaker at 100 rpm. Petri dishes and Erlenmeyer flasks containing 10 explants and 10 replicates were used.

Influence of TRIA concentration on DSE

In a second experiment, leaf explants were cultured in half strength MS medium supplemented with 100 mg.l^{-1} myo-inositol, 3.2 mg.l^{-1} pyridoxine, 10 mg.l^{-1} thiamine, 20 g.l^{-1} sucrose and 7 g.l^{-1} agar (Giridhar *et al.* 2004). Five treatments were used to evaluate the effect of combining BAP, IAA and TRIA at different concentration (μM) as follows: **A**: 1.1 BAP, 2.85 AIA, 0 TRIA; **B**: 1.1 BAP, 2.85 AIA, 4.55 TRIA; **C:** 1.1 BAP, 2.85 AIA, 11.38 TRIA; **D**: 1.1 BAP, 0 AIA, 4.55 TRIA and **E**: 1.1 BAP, 0 AIA, 11.38 TRIA. All media were adjusted to pH 5.6 before autoclaving for 21 min at 121 °C and 1.07 kg cm^{-2}. Petri dishes with 20 ml of culture media were used and explants were maintained in the dark at 26 ± 2°C. As before, Petri dishes containing 10 explants and 10 replicates were used. Weekly evaluations to record the number of embryos per explant were performed and the percentage of explants with somatic embryos was calculated using the formula [(number of explants that produced embryos / the total number of explants) x 100)].

Regeneration of somatic embryos

Caturra and Catuaí globular stage somatic embryos obtained in the experiments described above were transferred to the Yasuda *et al.* (1985) culture medium. Somatic embryos were cultivated in baby food jars, closed with with polyethylene food wrap (Glad, Costa Rica), at 26±2°C under 16 h light photoperiod (30 μmol.m^{-2}.s^{-1}). The germination percentage [(number of germinated somatic embryos / the total number of somatic embryos) x 100)] was recorded after four weeks of culture.

Statistical analysis

The statistical analysis was performed using one-way ANOVA and the significance of differences among treatment means were contrasted with Tukey's Honestly Significant Difference Test (HSD) at p=0.05. The program STATISTICA (StatSoft, Tulsa, OK, USA) version 6.0 was used.

RESULTS

DSE induction started with the development of a creamy compact non-embryogenic callus (scar tissue) on the cut edge of the leaf of Caturra and Catuaí varieties after 2 weeks of culture. These calli did not show continuous growth but globular and torpedo somatic embryos developed on their surface after 10 weeks of culture (Figure 1A).

The influence of TRIA concentration on DSE induction in both varieties was observed

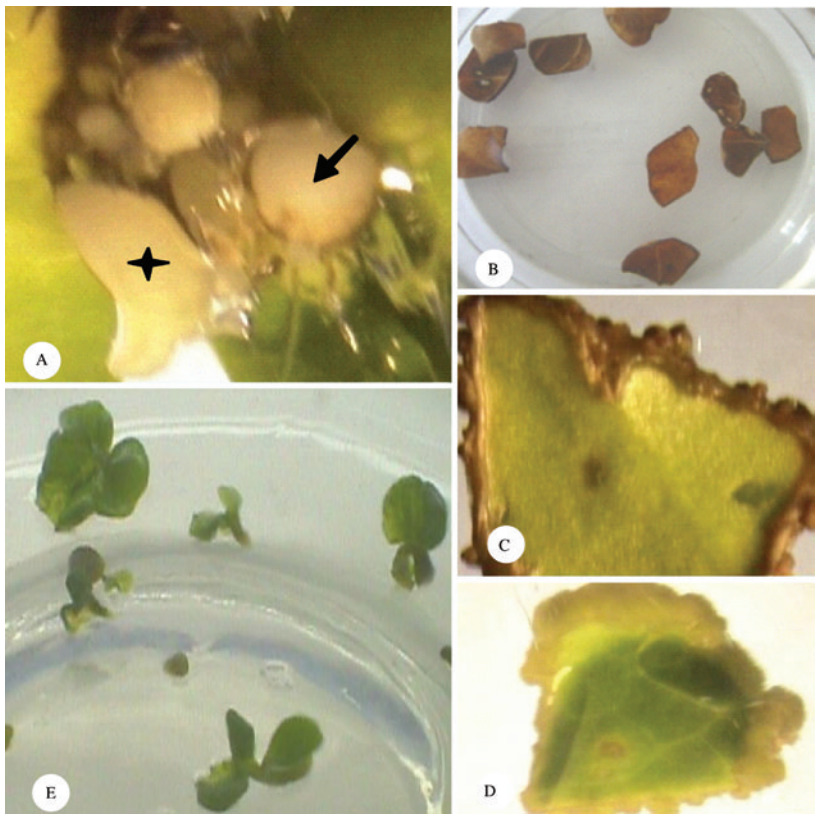

Fig. 1. Direct somatic embryogenesis from coffee leaves (*Coffea arabica* L. cv. Caturra and Catuaí). A. Globular (arrow) and torpedo somatic embryos (✦) formed using semisolid Yasuda *et al*. (1985) and medium containing TRIA. B. Non-direct somatic embryogenesis induction using Yasuda *et al*. (1985) liquid medium. C. Calli formed on the explants cultured under continuous white light and 16 h light photoperiod. D. Calli formed on the explants cultured in the dark. E. Coffee plantlets regenerated from somatic embryos culture on Yasuda *et al*. (1985) medium.

(Table 1). In treatment A (1.1 μM BAP and 2.85 μM AIA) low average of somatic embryos per explant and percentage of explants producing embryos were obtained compare to the results in treatment B, in which 4.55 μM TRIA was added (Table 1). The presence of TRIA significantly increased the average of embryos per explant and the percentage of explants producing embryos. Nevertheless, a higher concentration of TRIA in treatment C and E (11.38 μM) had a negative effect as no somatic embryos were produced in any of the studied varieties after 25 weeks of culture. The addition of 4.55 and 11.38 μM TRIA alone with 1.1 μM BAP in the culture medium did not improve DSE induction (treatment D and E). A positive interaction on DSE induction was observed by combining IAA and TRIA. Also, A significant increase on the average of somatic embryos per explant was observed in treatment B combining 1.1 μM BAP, 2.85 μM IAA and 4.55 μM TRIA.

The statistical analysis showed that higher average of embryoids per explant and higher percentage of explants with embryoids were obtained when the explants were cultured on semisolid Yasuda *et al*. (1985) medium. On the other hand, no embryogenic structures were observed in both varieties explants after 12 weeks

of culture on liquid Yasuda *et al*. (1985) medium (Table 2, Figure 1B). Additionally, using semi-solid media Catuaí exhibit a significant higher number of somatic embryos per explant than Caturra (p=0.05).

When examining the influence of light conditions on DSE induction in Caturra and Catuaí varieties, significant statistical differences were observed among the 3 light conditions evaluated. For both varieties, the average of embryos per explant and the highest percentage of explants with somatic embryos were obtained from explants cultured on semisolid Yasuda *et al*. (1985) medium, in the dark and under a 16 h light photoperiod (Table 3). The lowest average of embryos per explant and percentage of explants with somatic embryos were obtained using continuous white light (Table 3). In addition, browning of the leaf lamina and calli was observed on Caturra and Catuaí explants, cultured under continuous white light and 16 h light photoperiod (Figure 1C). In contrast, the calli formed on the Caturra and Catuaí explants cultured in the dark remained yellow throughout the evaluation period (Figure 1D).

Approximately 70-80% of Caturra and Catuaí globular somatic embryos developed into plantlets after 4 weeks of culture on Yasuda *et al*. (1985) medium (Figure 1E).

DISCUSSION

Investigation of the critical aspects of somatic embryogenesis is necessary in order to establish

Table 1. Effect of the TRIA concentration on direct somatic embryogenesis from leaf explants of Caturra and Catuaí vitro-plants after 25 weeks of culture on medium supplemented with different concentrations of BAP, IAA, TRIA.

Treatments		Number of somatic embryos per explant (mean±SE)		Explants producing somatic embryos (%)	
	BAP+IAA+TRIA (μM)	Caturra	Catuaí	Caturra	Catuaí
A	1.1+2.85+0	0.7 ± 0.2 b	1.6 ± 0.3 b	30.0 ± 6.6 b	44.0 ± 7.1 a
B	1.1+2.85+ 4.55	3.9 ± 0.5 a	3.6 ± 0.5 a	72.0 ± 6.4 a	62.0 ± 6.9 a
C	1.1+2.85+11.38	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c
D	1.1+0+4.55	0.1 ± 0.1 b	0.3 ± 0.2 c	2.0 ± 2.0 c	8.0 ± 3.9 b
E	1.1+0+11.38	0.0 ± 0.0 b	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 c

Values followed by the same letter are not significantly different with the Tukey HSD test at p=0.05.

Table 2. Effect of culture medium consistency on direct somatic embryogenesis induction from leaf explants of Caturra and Catuaí vitroplants after 12 weeks of culture Yasuda *et al*. (1985).

Variety	Consistency of culture media	Number of somatic embryos per explant in average (mean±SE)	Explants with somatic embryos (%)
Caturra	Semisolid	3.2 ± 0.2 b	61.0 ± 2.8 a
	Liquid	0 ± 0 c	0 ± 0 b
Catuaí	Semisolid	6.0 ± 04 a	68.0 ± 2.7 a
	Liquid	0 ± 0 c	0 ± 0 b

Values followed by the same letter are not significantly different with the Tukey HSD test at p=0.05.

Table 3. Effect of the light conditions on direct somatic embryogenesis induction from leaf explants of Caturra and Catuaí vitroplants after 12 weeks of culture on Yasuda *et al*. (1985).

Light condition	Caturra		Catuaí	
	Number of somatic embryos per explant (mean ± SE)	Explants with somatic embryos (%)	Number of somatic embryos per explant (mean ± SE)	Explants with somatic embryos (%)
Continuous light	1.7 ± 0.3 b	37.0 ± 4.9 b	4.3 ± 0.6 b	55.0 ± 5.0 b
Photoperiod	3.8 ± 0.5 a	70.0 ± 4.7 a	7.6 ± 1.0 a	68.0 ± 4.7 a b
Darkness	4.2 ± 0.4 a	76.0 ± 4.3 a	6.2 ± 0.6 a b	80.0 ± 3.8 a

Values followed by the same letter are not significantly different with the Tukey HSD test at p=0.05.

reliable protocols for the successful production and stable cultures of somatic embryos (Takanori and Cuello 2005). The induction and expression of coffee somatic embryos are influenced by the culture medium composition, the physical environment of the culture, the genotype, and the source and age of the explant (Fuentes *et al*. 2000). The results obtained in this research corroborated previous investigations in *Coffea* sp. concerning the effect of TRIA concentration, light conditions and culture media consistency on DSE induction. In Costa Rica, Arabica type coffee is grown extensively with 80% of the area being cultivated with Caturra and Catuaí varieties (Zamora 1997). Moreover, in Latin America, Arabica type coffee is responsible for all the coffee production (Kumar *et al*. 2006).

TRIA is an endogenous plant primary alcohol that enhances growth and yield in several annual vegetative and agronomic crop and forest species. TRIA increases ATP production in rape (*Brassica napus* L.) and rice (*Oryza sativa* L), and also increases photosynthesis in rice, maize (*Zea mays*) and peas (*Pisum sativum L*.) Ries (1991). On the other hand, Hangarter and Ries (1978) demostrated that TRIA increased *in vitro* growth of tobacco (*Nicotiana tabacum*) haploid cells and the fresh weight of cell cultures of tomato (*Lycopersicon esculentum*), potato (*Solanum tuberosum*), barley (*Hordeum vulgare* x *H. jubatum*), and beans (*Phaseolus vulgaris*).

Regarding TRIA influence on DSE, the highest average of somatic embryos per Caturra and Catuaí explant was obtained in a medium containing 1.1 μM BAP, 2.85 μM IAA, and 4.55 μM TRIA (Table 1). This is the first report in which the beneficial influence of TRIA in combination with IAA on DSE induction using Caturra and Catuaí leaves has been documented. In this regard, Tantos *et al*. (2001) reported that the effect of TRIA on rooting of *Malus domestica* cv. JTE-E4 and *Cerasus fruticosa* cv. Probocskai was improved when TRIA was combined with IBA.

Previously, Giridhar *et al*. (2004) developed a highly reproducible method for DSE induction in *C. canephora* cv. 274 and *C. arabica* cv. Hemavathy using leaf and stem segments. These authors showed that 11.38 μM TRIA in half strenght MS medium complemented only with 1.1 μM BAP induced the highest percentage of explants producing embryos (100%) in both species. Moreover, these authors reported a superior induction of somatic embryogenesis in explants coming from *C. arabica* (280±9.6) than those produced on explants from *C. canephora* (58.6±10.3). In contrast to Giridhar *et al*. (2004), in the present study 11.38 μM TRIA did not induce DSE on Caturra and Catuaí leaf segments. Probably, differences in cultivars, physiological state of the leaf, explant age and month of explant collection may account for the results obtained in both studies.

Although several specific responses have been demonstrated for TRIA, some of them are difficult to explain since TRIA elicit these responses indirectly (Ries 1991). Efforts have been made to elucidate the mode of action of TRIA (Chen *et al*. 2002). It has been demostrated that TRIA elicit the second messengers, OCTAM and TRIM, in the root tissue of rice (*Oryza sativa L*) seedlings. TRIM has been identified as 9-β-L (+)-adenosine, a naturally occurring growth substance, that is active in pico molar concentrations and moves rapidly throughout the plant (Ries *et al*. 1990, Ries, 1991). Furthermore, TRIA influences enzymes related to carbohydrate metabolism (Ries and Houtz 1983) and growth processes (Ries *et al*. 1977, Ries *et al*. 1990). Chen *et al*. (2002) isolated and characterized the genes up and down-regulated by TRIA and demonstrated that TRIA up-regulates the photosynthetic process and suppresses stress in rice plants.

It is postulated that TRIA increases the ratio of L (+)- to D(-)- adenosine at the tonoplast and the most probable source of adenosine is AMP, derived from ADP or ATP (Ries 1991). This increase in adenosine could promote *de novo* synthesis of cytokinins, which, in conjuntion with auxins, promotes cell division and triggers plant morphogenesis. Nevertheless, to test this hypothesis, it will be necessary to demonstrate that exogenously supplied TRIA causes an increase of cytokinin levels in plant cells. Moreover, it is possible that TRIA up or down regulates genes involved in somatic embryogenesis induction. However, to show this it will be necessary to isolate and characterize these genes and elucidate the molecular mechanisms for TRIA action on DSE.

The influence of environmental factors on somatic embryogenesis is an aspect that requires a careful consideration (Takanori and Cuello 2005). The results obtained in this investigation corroborated that DSE induction in *Coffea arabica* L. cvs. Caturra and Catuaí is influenced by the light conditions. Although, *C. arabica* and *C. canephora* DSE induction has been conducted in the dark (Molina *et al*. 2002), and under 14 h or 16 h light photoperiod (Yasuda *et al*. 1985, Hatanaka *et al*. 1991, Fuentes *et al*. 2000, Fuentes-Cerda *et al*. 2001, Quiroz-Figueroa *et al*. 2002), comparative studies on the influence of light conditions on coffee somatic embryogenesis have not been carried out before.

The highest average of somatic embryos per explant was obtained in the dark and a 16 h light photoperiod, instead of continuous white light (Table 2). In this sense, darkness is best for somatic embryogenesis induction, whereas light quality and intensity have effect on growth and development of somatic embryos (Takanori and Cuello 2005). The condition and intensity of light have been reported to affect somatic embryogenesis in terms of induction and growth of somatic embryos on *Glycine max* (Lazzeri *et al*. 1987), *Cydonia* L. (D'Onofrio *et al*. 1998), *Cydonia oblonga* (Morini *et al*. 2000), *Araujia sericifera* (Torné *et al*. 2001) and *Daucus carota* (Takanori and Cuello 2005).

DSE induction has been conducted using semisolid culture media on *C. arabica* cv. Typica (Yasuda *et al*. 1985), *C. arabica* cv. Caturra rojo (Bieysse *et al*. 1993, Fuentes-Cerda *et al*. 2001), F3, F4 and F5 generations of the cross between *C. arabica* cv. Caturra and the Timor hybrid (Molina *et al*. 2002), and *C. canephora* (Hatanaka *et al*. 1991, Fuentes *et al*. 2000). Recently, Canche-Moo *et al*. (2006) reported DSE induction from *C. canephora* cv. Robusta leaves using Yasuda *et al*. (1985) liquid culture medium. Moreover, DSE in liquid induction media has been reported in *Dianthus caryophyllus* L. (Yantcheva *et al*. 1998) and *Medicago* sp. (Denchev *et al*. 1991). In contrast to the results obtained by Canche-Moo *et al*. (2006), in the present work it was not possible to induce DSE from Caturra and Catuaí leaves using Yasuda *et al*. (1985) liquid culture media. In this regard, it has been reported that *C. canephora* genotypes show a better response to somatic embryogenesis than *C. arabica* (Van Boxtel and Berthouly 1996).

The fact that the protocol described in this work reduces the culture time required, makes it more time and cost effective. Moreover, it could reduce the somaclonal variation frequency observed with current indirect somatic

embryogenesis protocols since callus formation is not require, a process that takes approximately 2-4 months (Etienne and Bertrand 2001, Barry-Etienne *et al*. 2002ab, Etienne and Bertrand 2003). Finally, it will be interesting to validate this DSE protocol with wild coffee trees and other economically important varieties.

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LITERATURED CITED

BARRY-ETIENNE D., BERTRAND B., VASQUEZ N., ETIENNE, H. 2002a. Comparison of somatic embryogenesis derived coffee (*Coffea arabica* L.) plantlets regenerated *in vitro* or *ex vitro*: morphological, mineral and water characteristics. Ann. Bot. 90:77-85.

BARRY-ETIENNE D., BERTRAND B., SCHLÖNVOIGT A., ETIENNE H. 2002b. The morphological variability within a population of coffee somatic embryos produced in a bioreactor, affects the regeneration and the development of plants in nursery. Plant Cell, Tissue and Organ Culture 68:153-162.

BIEYSSE D., GOFFLOT A., MICHAUX-FERRIÉRE N. 1993. Effect of experimental conditions and genotypic variability on somatic embryogenesis in *Coffea arabica*. Can J Bot. 71:1496-1502.

CANCHEE-MOO R.L.R., KU-GONZÁLEZ A., BURGEFF C., LOYOLA-VARGAS V.M., RODRÍGUEZ-ZAPATA L.C., CASTAÑO E. 2006. Genetic transformation of *Coffea canephora* by vacuum infiltration. Plant Cell, Tissue and Organ Culture. 84:373-377.

CHEN X., YUAN H., CHEN R., ZHU L., DU B., WENG Q., HE G. 2002. Isolation and characterization of triacontanol-regulated genes in rice (*Oryza sativa* L.): possible role of triacontanol as plant growth stimulator. Plant Cell Physiol. 43:869-876.

DENCHEV P., VELCHEVA M., ATANASSOV A. 1991. A new approach to direct somatic embryogenesis in *Medicago*. Plant Cell Rep. 10:338-341.

D'ONOFRIO C., MORINI S., BELLOCCHI G. 1998. Effect of light quality on somatic embryogenesis of quince leaves. Plant Cell, Tissue and Organ Culture. 53:91-98.

ETIENNE H., BERTRAND B. 2001. Trueness-to-type- and agronomic characteristics of *Coffea arabica* trees micropropagated by the embryogenic cell suspension technique. Tree Physiol. 21:1031-1038.

ETIENNE H., BERTRAND B. 2003. Somaclonal variation in *Coffea arabica:* effects of genotype and embryogenic cell suspension age on frequency and phenotype of variants. Tree Physiol. 23:419-426.

FERNÁNDEZ-DA SILVA R., MENÉNDEZ-YUFFÁ A. 2003. Transient gene expression in secondary somatic embryos from coffee tissues electroporated with the gene *gus* and *bar*. Electron J Biotechnol. 6:29-38.

FRATERNALE D., GIAMPERI L., RICCI D., ROCCHI M.B.L., GUIDI L., EPIFANIO F., MARCOTULLIO M.C. 2003. The effect of TRIA on micropropagation and on secretory system of *Thymus mastichina*. Plant Cell, Tissue and Organ Culture 74:87-97.

FUENTES S., CALHEIROS M., MANETTI-FILHO J., VIEIRA L. 2000. The effects of silver nitrate and different carbohydrate sources on somatic embryogenesis in *Coffea canephora*. Plant Cell, Tissue and Organ Culture 60:5-13.

FUENTES-CERDA C.F.J., MONFORTE-GONZÁLEZ M., MÉNDEZ-ZEEL M., ROJAS-HERRERA R., LOYOLA-VARGAS V.M. 2001. Modification of the embryogenic response of *Coffea arabica* by the nitrogen source. Biotechnol Lett. 23:1341-1343.

GATICA A.M., ARRIETA G., ESPINOZA A.M. 2007. Comparison of three *in vitro* protocols for direct somatic embryogenesis and plant regeneration of *Coffea arabica* L. cvs. Caturra and Catuaí. Agronomía Costarricense 31:85-94.

GIRIDHAR P., INDU E.P., RAVISHANKAR G.A., CHANDRASEKAR A. 2004. Influence of TRIA on somatic embryogenesis in *Coffea arabica* L. and *Coffea canephora* P. EX FR. In vitro Cell Dev Biol Plant 40:200-203.

HANGARTER R., RIES S. 1978. Effect of TRIA on plant cell cultures *in vitro*. Plant Physiol. 61:855-857.

HATANAKA T., ARAKAWA O., YASUDA T., UCHIDA N., YAMAGUCHI T. 1991. Effect of plant growth regulators on somatic embryogenesis in leaf cultures of *Coffea canephora*. Plant Cell Rep. 10:179-182.

JIMÉNEZ V. 2001. Regulation of *in vitro* somatic embryogenesis with emphasis on the role of endogenous hormones. Rev Bras Fisiol Veg. 13:196-223.

JIMÉNEZ V. 2005. Involvement of plant hormones and plant growth regulators on *in vitro* somatic embryogenesis. Plant Growth Regul. 47:91-110.

KUMAR V., MADHAVA N., RAVISHANKAR G.A. 2006. Developments in coffee biotechnology-*in vitro* plant propagation and crop improvement. Plant Cell, Tissue and Organ Culture 87: 49-65.

LAZZERI P.A., HILDEBRAND D.F., COLLINS G.B. 1987. Soybean somatic embryogenesis: effects of nutritional, physical and chemical factors. Plant Cell, Tissue and Organ Culture 10:209-220.

LOYOLA-VARGAS V.M., FUENTES-CERDA C.F.J., MONFORTE-GONZÁLEZ M., MÉNDEZ-ZEEL M., ROJAS-HERRERA R., MIJANGOS-CORTES J. 1999. Coffee tissue culture as a new model for the study of somaclonal variation. *In*:18 th International Scientific Colloquium on Coffee. ASIC, Helsinki. p. 302-307.

MALABADI R., MULGUND G., NATARAJA K. 2005. Effect of TRIA on the micropropagation of *Costus speciosus* (koen.) Sm. using rhizome thin sections. In vitro Cell. Dev. Biol.-Plant. 41:129–132.

MOLINA D., APONTE M., CORTINA H., MORENO G. 2002. The effect of genotype and explant age on somatic embryogenesis of coffee. Plant Cell, Tissue and Organ Culture 71:117-123.

MOREL G. 1965.Clonal propagation of orchids by meristems culture. Cymbidium Soc News 20:3.

MORINI S., D'ONOFRIO C., BELLOCCHI G., FISICHELLA M. 2000. Effect of 2,4-D and light quality on callus production and differentiation from *in vitro* cultured quince leaves. Plant Cell, Tissue and Organ Culture 63:47- 55.

MURASHIGE T., SKOOG F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant. 15:473-497.

QUIROZ-FIGUEROA F.R., FUENTES-CERDA C.F.J., ROJAS-HERRERA R., LOYOLA-VARGAS V.M. 2002. Histological studies on the developmental stages and differentiation of two different somatic embryogenesis systems of *Coffea arabica*. Plant Cell Rep. 20:1141-1149.

RAEMAKERS C.J.J.M., JACOBSEN E., VISSER R.G.F. 1995. Secondary somatic embryogenesis and applications in plant breeding. Euphytica 81:93-107.

REDDY B.O., GIRIDHAR P., RAVISHANKAR G.A. 2002. The effect of TRIA on micropropagation of *Capsicum frutescens* and *Decalepis hamiltonii* W&A. Plant Cell, Tissue and Organ Culture 71:253-258.

RIES S., WERT V.F., SWEELEY C.C., LEAVITT R.A. 1977. TRIA: a new naturally occurring plant growth regulator. Science 195:1339–1341.

RIES S., HOUTZ R. 1983. TRIA as a plant growth regulator. HortScience 18:654-662.

RIES S., WERT V., O´LEARY N.F.D., NAIR M. 1990. 9-β-L(+)-adenosine: a new naturally occurring plant growth substance elicited by triacontanol in rice. Plant Growth Regul. 9:263-273.

RIES S. 1991. TRIA and its second messenger 9-β-L(+)-adenosine as plant growth substances. Plant Physiol. 95:986-989.

TAKANORI H., CUELLO J. 2005. Regulating radiation quality and intensity using narrow-bands leds for optimization of somatic embryogenesis. Proceedings of the 2005 Annual Meeting of the American Society of Agricultural Engineers.

TANTOS A., MÉSZÁROS A., FARKAS T., SZALAI J., HORVÁTH G. 2001. TRIA-supported micropropagation of woody plants. Plant Cell Rep. 20:16-21.

TORNÉ J.M., MOYSEE L., SANTOS M., SIMÓN E. 2001. Effect of light quality on somatic embryogensesis in *Araujia sericifera*. Physiol Plant. 111:405.

YANTCHEVA A., VLAHOVA M., ATANASSOV A. 1998. Direct somatic embryogenesis and plant regeneration of carnation (*Dianthus caryophyllus* L.). Plant Cell Rep. 18:148-153.

YASUDA T., FUJII Y., YAMAGUCHI T. 1985. Embryogenic callus induction from *Coffea arabica* leaf explant by benziladenine. Plant Cell Physiol. 26:595-597.

VAN BOXTEL J., BERTHOULY M. 1996. High frequency somatic embryogenesis from coffee leaves: factors influencing embryogenesis, and subsequent proliferation and regeneration in liquid medium. Plant Cell, Tissue and Organ Culture 44:7-17.

ZAMORA L. 1997. Costa Rica. Un enfoque de caficultura sostenible para Costa Rica. *In*: Panel de agricultura sostenible: XVIII Simposio Latinoamericano de Caficultura. IICA/PROMECAFE. EDITORAMA. Costa Rica. p. 85-99.