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DETERMINATION OF CALLUS GROWTH CURVE IN CONILON COFFEE[1]

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ABSTRACT - Callus culture has shown a great potential for large-scale multiplication of superior genotypes in a short period of time. The objective of this work was to establish a methodology to induce callus formation in leaf fragments of *Coffea canephora* Conilon cultivar, to determine the growth curve and to analyze the development of the calli. The growth curve allows to determine the suitable time to subculture the calli in a new medium. The explants were inoculated in 50% MS medium with 10 $mg.L^{-1}$ thiamine, 1 $mg.L^{-1}$ pyridoxine, 1 $mg.L^{-1}$ nicotinic acid, 1 $mg.L^{-1}$ glycine, inositol 100 $mg.L^{-1}$, hydrolysed casein 100 $mg.L^{-1}$ and 400 $mg.L^{-1}$ malt extract, 20 $g.L^{-1}$ sucrose, 8 $g.L^{-1}$ agar, supplemented with IBA (10 μM), 2,4-D (20 μM) and 2iP (10 μM). To determine the growth curve, the calli were weighted up to the 60th day of culture. The callus growth curve presented sigmoidal shape, with five distinct phases. The subculture must be done on the 53th day of culture, aiming at the plant regeneration.

Keywords: *Coffea canephora*. Callogenesis. Growth regulators.

DETERMINAÇÃO DA CURVA DE CRESCIMENTO DE CALOS EM CAFÉ CONILON

RESUMO - A cultura de calos tem mostrado grande potencial para multiplicação em larga escala de genótipos superiores e em curto espaço de tempo. O objetivo deste trabalho foi induzir a formação de calos em explantes foliares de *Coffea canephora* var. Conilon, e avaliar o desenvolvimento dos calos, determinando sua curva crescimento. A curva de crescimento de calos permite inferir sobre o momento adequado para subcultivá-los em novo meio de cultura. Os explantes foram inoculados em meio MS 50% com 10 $mg.L^{-1}$ de tiamina, 1 $mg.L^{-1}$ de piridoxina, 1 $mg.L^{-1}$ de ácido nicotínico, 1 $mg.L^{-1}$ de glicina, 100 $mg.L^{-1}$ de inositol, 100 $mg.L^{-1}$ de caseína hidrolisada e 400 $mg.L^{-1}$ de extrato de malte, 20 $g.L^{-1}$ de sacarose, 8 $g.L^{-1}$ de ágar e acrescido de AIB (10 μM), 2,4-D (20 μM) e 2iP (10 μM). Para determinar a curva de crescimento, calos foram pesados até o 60^{o} dia de cultivo. A curva de crescimento dos calos seguiu um padrão sigmóide, com cinco fases distintas. O subcultivo deve ser feito no 53^{o} dia, visando à regeneração de plantas.

Palavras-chave: *Coffea canephora*. Calogênese. Reguladores de crescimento.

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INTRODUCTION

The Conilon cultivar belongs to the species *Coffea canephora* Pierre, and was taken to Rondônia state by the first immigrants from the Southeast of the country. Its product has more solubility than the *Coffea arabica* L. and can be very useful for the soluble coffee industries (FERRÃO et al., 2007). Other advantages of the cultivar are its relative resistence to many diseases that affect *C. arabica* such as rust, caused by the fungus *Hemileia vastatrix* Berk. & Br., and its good adaptation to the edaphic and climatic conditions of Rondônia. Moreover, *C. canephora* has high production capability, is resistant to the nematode *Meloidogyne exigua* Goeldi, tolerant to *M. incognita* Kofoid & White and, due to its well developed rooting system, is also resistant to the dryness (MARTINS et al., 2006).

All the mentioned characteristics are very interesting and useful in breeding programs. In *C. arabica*, the methods are restricted to those applied to self compatible plants. In *C. canephora*, due to its alogamic reproduction, several methods can be used (FERRÃO et al., 2007).

The cross fertilization in *C. canephora* results in non-uniform cultivations in relation to vegetative characteristics (height, architecture, angle of branches, shape and size of leaves), production (productivity, fruit size, shape and maturation) and susceptibility to diseases (PAULINO et al., 1985). This variability difficults the cultural treatments and reduces the general productivity.

The quality of Conilon coffee can be increased by micropropagation of selected mother genotypes (BRAGANÇA et al., 2001). The pioneer work on coffee micropropagation was published by Staritsky (1970), who was successful at callus induction in leaves from several species. After that, many studies have been made with different methods and species (MEZZETTI et al., 1991; LANDA et al., 2000; SERRA et al., 2000; SANTOS et al., 2008). Some described techniques are microcuttings, organogenesis, callogenesis, somatic embryogenesis and zygotic embryo culture (SANTANA-BUZZY et al., 2007).

Tissue culture techniques should minimize the necessary time for the introduction of new cultivars into the commercial market and so increase the availability of plants with improved horticultural characteristics (RÊGO et al., 2009). Moreover, these techniques allow the mass production of genetically superior plants in short periods of time (SOARES et al., 2009).

Callus induction is one of the most utilized techniques in the rescue of entire populations of induced mutants, from somaclonal variation or transgenic production. The establishment of these populations results on the development of new cultivars. For this, studies of every aspect (kind of explant, medium composition, light and temperature of incubation) involved on the calli growth and plant regeneration are needed (SANTOS et al., 2005; PINTO; LAMEIRA, 2001).

The regeneration can be defined as a process of vegetative multiplication that results on the obtention of an entire plant from only one cell (BELTRÃO et al., 2008). The knowledge over the cell division velocity allows to infer about the physiologic changes in the callus and consequently, aids to optimize the regeneration protocols (SERRA et al., 2000).

According to Santos et al. (1997), the calli growth curves are important to identify the stages of the fundamental growth processes, allowing to know the exact moment to subculture the calli in a new medium. These stages are: 1) lag phase: whitout cell multiplication, beginning of the metabolite mobilization, syntesis of proteins and specific metabolites; 2) exponential phase: cell division reach the maximum; 3) linear phase: reduction of the cell division velocity; 4) deceleration phase: cell division decreases and cell expansion occurs; and 5) stationary phase: no cell division or weight increasing (CASTRO et al., 2008).

The objective of this work was to induce callus in Conilon leaf fragments and to study the callus growth, establishing its growth curve, to make available information that can be useful in the callus manipulation and plant regeneration.

MATERIAL AND METHODS

The experiments took place at the Plant Tissue Culture Laboratory of the Brazilian Agricultural Research Corporation (Empresa Brasileira de Pesquisa Agropecuária) – Rondônia Branch, in Porto Velho.

Leaves from Conilon cultivar plants were collected in the second pair of plagiotropic branches. At the laboratory, they were washed with water and a detergent agent and immersed in alcohol 70% for 1 minute, NaOCl 1.25 for 30 minutes and rinsed three times in sterile distilled water. The leaves were cut into fragments of about 1.0 cm^2, which were inoculated in assay tubes with the adaxial face in contact with the medium. This medium was supplemented with half the concentration of salts of the MS medium (MURASHIGE; SKOOG, 1962) and tiamin 10 $mg.L^{-1}$, piridoxin 1 $mg.L^{-1}$, nicotinic acid 1 $mg.L^{-1}$, glicin 1 $mg.L^{-1}$, inositol 100 $mg.L^{-1}$, 100 casein $mg.L^{-1}$, malt extract 400 $mg.L^{-1}$, sucrose 20 $g.L^{-1}$, agar 8 $g.L^{-1}$ and the growth regulators IBA (10 μM), 2,4-D (20 μM) and 2iP (10 μM). A total number of 150 explants were inoculated, one per assay tube, and maintened in the darkness in a growth-chamber at 24±2 °C. In periods of 10 days, during 60 days, 20 calli were weighted. The experimental design was entirely randomized and the data were submited to regression analysis.

RESULTS AND DISCUSSION

The growth curve followed a sigmoidal pattern, with the lag, exponential, linear, deceleration and stationary phases (Figure 1). Similar results were obtained by Mesquita et al. (2003) with callus from *Litchi chinensis* Sonn leaves, by Lima et al. (2007) with callus from *Croton urucurana* Baill. leaves, by Santos et al. (2008) with callus from Apoatã cultivar of *C. canephora* leaves and stems and by Nogueira et al. (2008) with callus from *Byrsonima intermedia* A. Juss. leaves. Different results were obtained by Santos et al. (2003) with callus from Rubi cultivar of *C. arabica*, which observed only three phases (lag, exponential and linear).

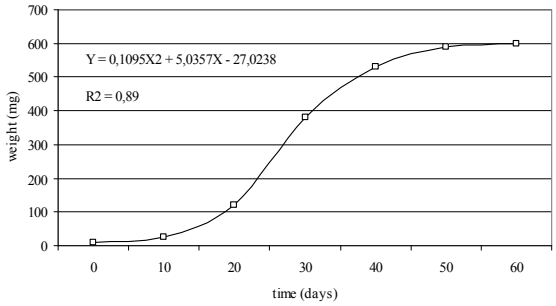

Figura 1. Growth curve of calli from *Coffea canephora* leaves during 60 days of culture.

The lag phase is characterized by accumulation of weight, whitout cell division. In this work the lag phase occurred from the beginning of the culture until the 15th day, with increase of 15 mg. According to Scragg and Allan (1993), the lag phase is a period of energy production. Landa et al. (2000) evaluated calli obtained from *Caryocar brasiliense* Camb. leaves and identified the lag phase until the 7th day. Santos et al. (2008) observed the calli growth from leaves and stems of Apoatã cultivar of *C. canephora* identifying the lag phase until the 28th and the 49th days, respectively. Santos et al. (2003) observed this phase in *C. arabica* Rubi cultivar until the 42th day, with 64% of growth. Mezzetti et al. (1991), evaluated calli growth from *Actinidia deliciosa* A. Chev. leaves with gain of fresh and dry matter until the 30th day. Serra et al. (2000) observed this phase until the 30th day in leaf calli of *Bertholletia excelsa* H.B.K.

The exponential phase is the period of maximum cell division and occurred from the 16th to the 34th day, with 390 mg of weight increase. Scragg and Allan (1993) referred to this phase as a biossintetic stage. In *Smilax japecanga*, this phase occurred from the 18th to the 26th day (SANTOS et al., 1997). In *Caryocar brasiliensis*, this phase was identified between the 7th and the 35th day (LANDA et al., 2000). Santos et al. (2003) observed this phase in *C. arabica* Rubi cultivar from the 42th to the 47th day, with 87% of growth. In *Bertholletia excelsa* H.B.K. this occurred from the 30th to the 53th day (SERRA et al., 2000).

The linear phase is characterized by the reduction in the cell division and the increase of cell volume (SERRA et al., 2000). This phase was identified from the 35th to the 42th day, with 540 mg of weight increase. In *C. arabica* Rubi cultivar this occurred between the 77th and the 84th day with 6% of growth (SANTOS et al., 2003). In *Bertholletia excelsa* H.B.K. this occurred from the 53th to the 60th day (SERRA et al., 2000). In *C. canephora* Apoatã cultivar, this phase was observed from the 63th to the 70th day (SANTOS et al., 2008).

Between the 43th and the 53th day, the deceleration phase was observed, with increase of 60 mg. In this period the calli must be transferred to a new medium, due to the nutrients reduction, the agar drying and the accumulation of toxic substances (SMITH, 1992).

Stationary phase started at the 54th day, when the secondary metabolites accumulate on the tissues and no cell division or weight increasing occurs.

According to the evaluation of the calli growth curve, the subculture must be done in the beginning of deceleration phase, i.e., on the 53th day of culture, aiming at the plant regeneration.

CONCLUSION

The calli growth curve presented sigmoidal shape, with five distinct phases. The subculture must be done on the 53th day of culture, aiming at the plant regeneration.

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