SCIENTIFIC NOTE

INDIRECT AND DIRECT SOMATIC EMBRYOGENESIS IN ROOT EXPLANT OF THE *COFFEA ARABICA* L. (RUBIACEAE)

IVANILDA DOS SANTOS ALVES

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Abstract: In the application of indirect and direct somatic embryogenesis in *Coffea arabica*, leaf explants are normally used. There are no reports in the literature on the use of root-type explants in somatic embryogenesis of this species. However, root-type explants are used to induce somatic embryogenesis in different species. The aim in this study was to verify the ability of indirect and direct somatic embryogenesis in *C. arabica* root explants. For this purpose, 1 cm long root explants obtained from *in vitro* plants of the cultivar Catuaí Vermelho IAC 81 were inoculated in means of induction of indirect and direct somatic embryogenesis, in the absence of light, at 25 °C. In the indirect way, the explants formed calluses and then somatic embryos. In the direct path, the root segments formed only somatic embryos and the explants did not show any change in their external morphology. The results obtained indicate that explants of the root type of *C. arabica* from the cultivar Catuaí Vermelho IAC 81 have the capacity to form somatic embryos by indirect and direct somatic embryogenesis.

Keywords: callus, 2,4-D, 2-iP, Kinetin, somatic embryo.

EMBRIOGÊNESE SOMÁTICA INDIRETA E DIRETA EM EXPLANTE DE RAIZ DE *COFFEA ARABICA* L. (RUBIACEAE)

Resumo: Na aplicação da embriogênese somática indireta e direta em *Coffea arabica* normalmente se utilizam explantes do tipo foliar. Na literatura não se verifica relatos do uso de explante do tipo raiz na embriogênese somática desta espécie. Mas, explantes do tipo raiz são utilizados para a indução da embriogênese somática em diferentes espécies. O objetivo neste estudo foi verificar a capacidade de embriogênese somática indireta e direta em explantes de raízes de *C. arabica*. Para tanto, explantes de raiz de 1 cm de comprimento obtidos de plantas *in vitro* da cultivar Catuaí Vermelho IAC 81 foram inoculados em meios de indução de embriogênese somática indireta e direta, em ausência de luz, a 25 °C. Na via indireta, os explantes formaram calos e em seguida embriões somáticos. Na via direta os segmentos de raiz formaram apenas embriões somáticos e os explantes não apresentaram qualquer alteração de sua morfologia externa. Os resultados obtidos indicam que explantes do tipo raiz de *C. arabica* da cultivar Catuaí Vermelho IAC 81 têm capacidade de formar embriões somáticos pela embriogênese somática indireta e direta.

Palavras-chave: calos, 2,4-D, 2-iP, cinetina, embrião somático.

Somatic embryogenesis can occur by direct or indirect pathways (Williams & Maheswaran, 1986). The species *Coffea arabica* L. can form somatic embryos by both vias. In this species, the indirect pathway occurs in two phases, the first being callogenesis to form a callus, which is a mass of cells that develop in the region on the explant cut (Sondhal & Sharp, 1977 and Almeida et al., 2008). The second phase consists of the induction and development of the somatic embryos as from cells from certain sectors of the callus. On the other hand, in the direct somatic embryogenesis the embryos are formed in a single phase without the formation of a callus (Almeida & Silvarolla, 2009; Almeida et al., 2016 and Alves et al., 2018).

The occurrence of the somatic embryogenesis can be affected by the choice of the type of explant (Molina et al., 2002 and Lópes-Gómes et al., 2010). In addition to this the application of somatic embryogenesis can also vary between different types of explant from the same plant (Máthé et al., 2011; Moon et al., 2013 and Venkataiah et al., 2016).

It should be mentioned that ever since pioneering studies were carried out with the species *C. arabica* (Sondhal & Sharp, 1977) somatic embryogenesis has normally been applied to foliar type explants (Yasuda et al., 1985, Pereira et al., 2007; Rezende et al., 2011 and Almeida et al., 2016). Although a leaf type explant has been used for *C. arabica* it will also be important to know whether other sources of explants of this same species could present somatic embryogenesis.

Root explant has been used for the application of somatic embryogenesis in different species (Paul & Sikdan, 2005; Flores et al., 2006; Akter & Al-Forkan, 2010; Zhou et al., 2010; Sharon et al., 2011; Yaacob et al., 2012; Scotton et al., 2013 and Konar et al., 2018. However, there are no reports in the literature on the application of somatic embryogenesis in a *Coffea* root explant. Thus, the objective in the present study was to verify the capacity of indirect and direct somatic embryogenesis in root explants of *C. arabica*.

In this study, *in vitro* plant roots of *C. arabica* cultivar Catuaí Vermelho IAC 81 were used. The *in vitro* plants had an average of ten pairs of leaves, most of them were physiologically mature, with a size between 10 to 17 mm long and 9 to 12 mm wide, and extensive roots and these were previously obtained by direct somatic embryogenesis (Almeida & Silvarolla, 2009). Approximately 1 cm long root explants were inoculated into culture media for the induction of direct and indirect somatic embryogenesis.

Due to the absence of reports on somatic embryogenesis in roots of *C. arabica*, in this study it was used those more mentioned for the induction of indirect and direct pathways on leaf explants of this species. The Murashige & Skoog (1962) (MS) culture medium, as modified by Söndhal & Sharp (1977) by the addition of Inositol (550 µM) and Cysteine (210 µM), was used for the induction of indirect somatic embryogenesis. The root explants were inoculated into the callogenesis induction medium and maintained as such for 120 days. After this period, the calluses formed were transferred to the somatic embryo induction medium.

For callogenesis, MS medium with the addition of 30 g/L sucrose, 2.5 µM 2,4 Dichlorophenoxyacetic acid and 5 µM Kinetin was used, and in the somatic embryo induction phase a culture medium with ½ the salts concentration of the MS medium was used with the addition of 20 g/L sucrose, 0.5 µM Naphthaleneacetic acid and 2.5 µM kinetin.

For the induction of direct somatic embryogenesis a culture medium with ½ the salts concentration of the MS medium was used with the addition of 20 $g.L^{-1}$ sucrose and 10 µM 2-Isopentenyladenine (2-iP) (Alves et al., 2018).

A culture medium with ½ the salts concentration of the MS medium and the addition of 20 $g.L^{-1}$ sucrose and no plant growth regulator was used for the germination of the somatic embryos obtained by both the indirect and direct pathways.

The pH values of all the culture media were adjusted to pH 5.8, and then jellified with 2 $g.L^{-1}$ Phytagel and autoclaved at 121 °C and 1.5 atm of pressure for twenty minutes. All the experiments were carried out at 25 °C in the absence of light.

The treatments were evaluated periodically for their capacity for somatic embryogenesis according to Almeida et al. (2008), as estimating of the size of the calluses and determining the number of somatic embryos formed. Each treatment consisted of ten repetitions each with four radicular explants, and a completely random experimental design was adopted. Data from callus size estimation and the number of somatic embryos formed were expressed as mean standard error of the media.

It was shown in the present study that root explants from *in vitro* plants of *C. arabica* cultivar Catuaí Vermelho IAC 81 presented the responses for both indirect (Fig. 1A) and direct (Fig. 1B) somatic embryogenesis. For indirect somatic embryogenesis, the first factor analyzed was the rate of root explants showing the formation of a callus throughout the 120 days of the experiment, which was from 80 to 100% for all explants (data not shown). The estimate of the size of the calluses formed by the root ex-

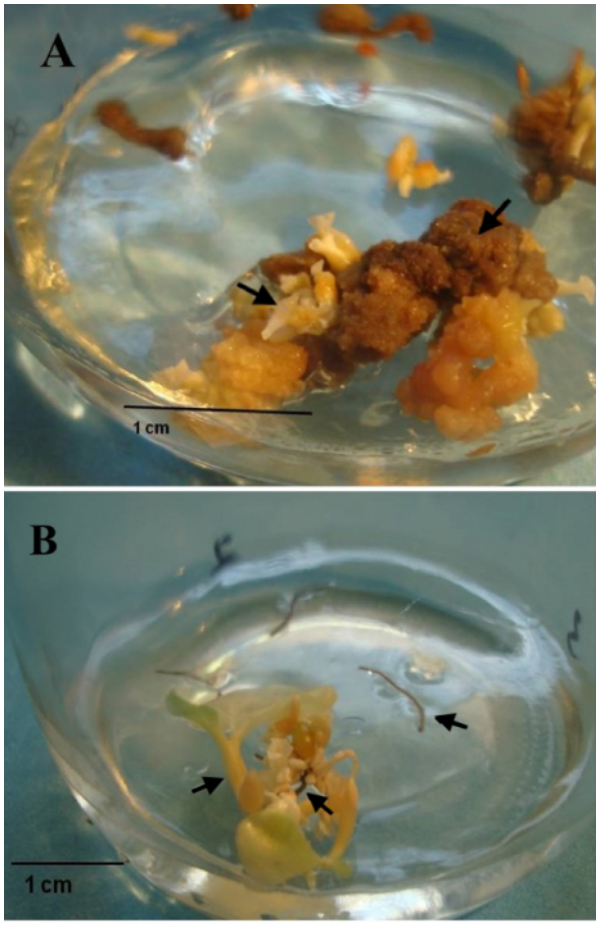

Fig. 1. Somatic embryogenesis from root explants of the cultivar Catuaí Vermelho IAC 81 of *Coffea arabica* L., maintained in the absence of light and at 25 °C. A. Indirect somatic embryogenesis: Callus (top arrow) formed from radicular explant with formation of somatic embryos (bottom arrow). B. Direct somatic embryogenesis root explant (right arrows) with somatic embryo formation (left arrow).

plants showed that they were below 4 mm in size 30 days after the start of the experiment, but after 120 days this response increased (Fig. 2A). When the calluses reached 120 days of age, with an average size of 14 mm, they were transferred to the embryogenesis induction medium and subsequently presented the formation of somatic embryos (Fig. 2B), that begins average 90 days but this remains low until the end of the experiment.

The cultivar Catuaí Vermelho IAC 81 root explants submitted to the process of direct somatic embryogenesis showed the formation of somatic embryos in a single culture medium, as can be seen in Fig. 1B. The root explants form somatic embryos 60 days after the beginning of the experiment and maintain an increase in production until the end of the evaluations. It was also observed that in the direct route, the root segments did not change their external morphology, except became oxidized and formed somatic embryos on their surface (Fig. 1B). Possibly, these explants can respond more efficiently in both embryogenesis pathways if they are subjected to different concentrations of plant growth regulators or other temperature and lighting conditions.

C. arabica root type explants showed a response pattern similar to that observed in leaf explants of the same species, with callogenesis

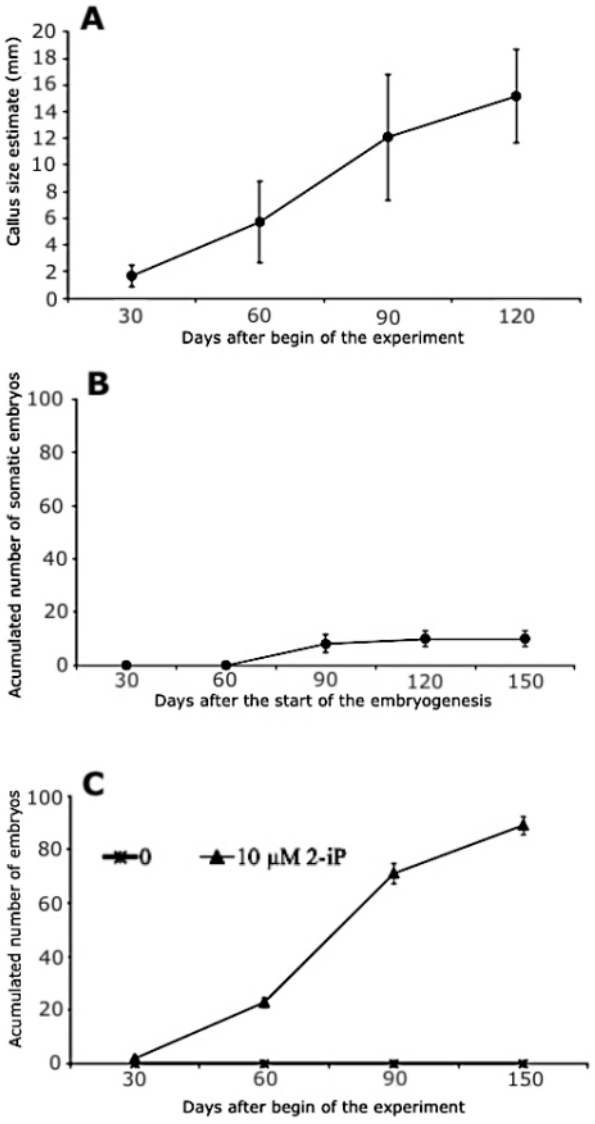

Fig. 2. Indirect and direct somatic embryogenesis from root explants of the cultivar Catuaí Vermelho IAC 81 of *Coffea arabica* L., maintained in the absence of light and at 25 °C. Indirect via: A. Calogenesis. B. Formation of somatic embryo from callus. Direct via: C. Root segment with formation of somatic embryos.

followed by the formation of somatic embryos (Almeida et al., 2008), whereas using the direct pathway the process occurred in a single culture medium, without the callogenesis phase (Almeida et al., 2016 and Alves et al., 2018). Taking into account the results obtained, it is noted that the formation of somatic embryos occurred at similar times in both pathways. Direct somatic embryogenesis proved to be more efficient than the indirect route due to the higher production of somatic embryos. However, these results are preliminary, there is a need for further studies, mainly regarding the concentration and type of plant growth regulator and also other types of protocols for a more precise conclusion on aspects such as the speed of the process and the production of somatic embryos. Thus, the results obtained in this study indicate that explants of the root type of *C. arabica* cultivar Catuaí Vermelho IAC 81 showed the capacity for both direct and indirect somatic embryogenesis.

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