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Osmotic potential intensity in the control of direct somatic embryogenesis in coffee tree

Abstract – The objective of this work was to characterize the effect of the osmotic potential of the culture medium on the control of direct somatic embryogenesis in coffee (*Coffea arabica*) tree. Leaf explants of the Mundo Novo cultivar and four hybrids were subjected to the direct route in the presence of polyethylene glycol 6000, at the following concentrations: 0, 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0%. Embryogenic structures and somatic embryos were formed at a threshold osmotic potential of up to -0.691 and -0.374 MPa, respectively, with a reduction or inhibition in these responses under a more negative osmotic potential.

Index terms: *Coffea arabica*, embryogenic structure, PEG 6000, somatic embryo.

Intensidade do potencial osmótico no controle da embriogênese somática direta em cafeeiro

Resumo – O objetivo deste trabalho foi caracterizar o efeito do potencial osmótico do meio de cultura no controle da embriogênese somática direta em cafeeiro (*Coffea arabica*). Explantes foliares da cultivar Mundo Novo e de quatro híbridos foram submetidos à via direta na presença de polietilenoglicol 6000, nas seguintes concentrações: 0, 2,5, 5,0, 7,5, 10,0, 12,5 e 15,0%. Estruturas embriogênicas e embriões somáticos formaram-se no potencial osmótico limite de até -0,691 e -0,374 MPa, respectivamente, com redução ou inibição destas respostas nos potenciais osmóticos mais negativos.

Termos para indexação: *Coffea arabica*, estrutura embriogênica, PEG 6000, embrião somático.

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Somatic embryogenesis is a plant biotechnological process for the micropropagation of different species through the formation of somatic embryos via indirect and direct routes (Horstman et al., 2017).

The response of the somatic embryogenesis of different species is affected by osmotic stress. In tissue in vitro, it can be induced by the polyethyleneglycol 6000 (PEG 6000) osmotic agent through water restriction (Merkle et al., 1995; Elmaghrabi et al., 2017), aiming to select genotypes with drought tolerance (Eliane et al., 2019) and to induce somatic embryos and their maturation, as well as callogenesis (Neofiti et al., 2020; Silveira et al., 2020).

The species of *Coffea arabica* L. responds to indirect or direct somatic embryogenesis or to both (Almeida et al., 2016), although it may show a reduced or no response under the direct route (Nic-Can et al., 2015). The osmotic stress has been more frequently applied in

indirect somatic embryogenesis, maybe it can too favor the direct route in coffee.

The objective of this work was to characterize the effect of the osmotic potential of the culture medium on the control of direct somatic embryogenesis in coffee tree.

The genetic material used was obtained from adult plants of the H8089 ('Catuaí Vermelho IAC-24' x Geisha IAC-1137), H8105 ('Catuaí Vermelho IAC-81' x BA10 IAC-1110), and H8427 ('Acaiá IAC-474-1' x BA10 IAC-1110) hybrids and from the Mundo Novo IAC 376-4 cultivar. The plants were grown at the farm of Instituto Agronômico de Campinas, located in the state of São Paulo, Brazil. The experiment was carried out in a completely randomized design, with eight treatments (doses of PEG 6000), with 13 replicates, with two explants in each. The treatments were evaluated for rate of formation of embryogenic structures at 120 days after cultivation and number of somatic embryos formed at 210 days.

In the morning, coffee leaves were plucked up to the third pair of leaves from plagiotropic twigs belonging to the middle region of the tree canopy and were, then, disinfected as described in Almeida et al. (2016). Rectangular explants of 1.5×2.0 cm were obtained from these leaves, excluding the areas of the main rib, the basal and apical portions, and the edges. The explants were inoculated by placing the adaxial epidermis facedown on the surface of the used culture medium (30 mL), maintained in transparent 150 mL glass flasks and in the dark, at 25ºC, for a mean period of 210 days.

Direct somatic embryogenesis was induced in a culture medium containing half the concentration of Murashige & Skoog (MS) (Murashige & Skoog, 1962) salts, plus 20 g L^{-1} sucrose and 10 µM of 2-isopentenyladenine. Then, pH was adjusted to 5.8, jellified with 5.0 g L^{-1} agar or 2.0 g L^{-1} Phytagel and autoclaved at 121ºC and 1.5 atm for 20 min. PEG 6000 was added to the culture medium at the concentrations of 0, 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0%. Osmotic potential was determined using an osmometer both for the culture medium samples with and without the addition of PEG 6000.

The somatic embryos formed by the explants were transferred to the germination medium – consisting of half the MS salts plus 20 g L^{-1} sucrose, without the plant growth regulator – and maintained at 25ºC in the light. After 30 days, they were transferred to a same culture medium until reaching plant size at approximately 90 days.

The results related to the percentage of explants with the formation of embryogenic structures were subjected to analyses of variance and Tukey's test, at 5% probability, in order to compare means using the GENES statistic software (Cruz, 2006). To determine the osmotic potential induced by PEG 6000, three samples of the culture medium were used, and the obtained data were subjected to the linear regression analysis.

As the concentration of the osmotic agent was increased, the osmotic potential of the culture medium became more negative (Figure 1). The culture medium without PEG 6000 also showed a negative osmotic potential, which was more negative in the medium jellified with agar than in that with Phytagel, with respective values of -0.534 and -0.374 MPa.

Although the leaf explants of the four genotypes subjected to the direct route formed embryogenic

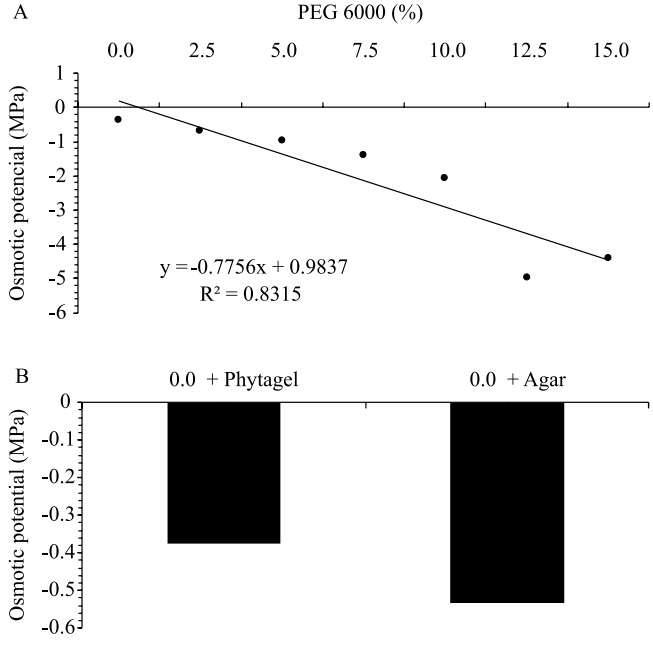

Figure 1. Osmotic potential of culture medium for the induction of the direct somatic embryogenesis of coffee (*Coffea arabica*) genotypes with the addition of different concentrations of the osmotic agent polyethyleneglycol 6000 (PEG 6000) and gelling with 2.0 g L^{-1} Phytagel (A) or 5.0 g L^{-1} agar (B).

structures, their responses depended on PEG 6000 concentration and genotype (Figure 2). In general, as the concentration of PEG 6000 increased, the rate of formation of the embryogenic structures decreased, being higher at the concentrations of 0, 2.5, and 5.0% PEG 6000, especially at 2.5%, and lower at 7.5 and 10.0%. None of the genotypes, however, showed any response at the concentrations of 12.5 and 15.0%. In the treatment without the osmotic agent, most genotypes showed higher rates of embryogenic structure formation than those that received PEG 6000; the exception was the H8427 genotype, with the highest response at the concentration of 2.5%, which did not differ significantly from that of the control jellified with agar (Figure 2).

In the treatments with PEG 6000, the genotypes did not develop somatic embryos, except cultivar Mundo Novo, which formed two of them at the concentration of 2.5%. Since somatic embryo formation was reduced or non-existent in the evaluated genotypes, the used PEG 6000 concentrations represented a stress factor for the direct somatic embryogenesis of this species. In the culture media without the addition of the osmotic agent, however, somatic embryos were formed, with a varying response depending on the used genotype and solidifying agent. The highest numbers of somatic embryos were formed by H8089, being 213 and 73 in the presence of Phytagel and agar, respectively; followed by those of the Mundo Novo cultivar, with 10 embryos in Phytagel and 18 in agar; of H8105, which only responded in the treatment with Phytagel; and of H8427, with just one embryo in agar.

Also in the treatments with PEG 6000, the intensity of the osmotic potential varied. The osmotic potential threshold for the initiation of embryogenic structures was up to -2.07 MPa and, for the formation of somatic embryos, up to -0.691 MPa, at 10.0 and 2.5% PEG 6000, respectively. However, all responses were inhibited completely at -4.971 and -4.386 MPa at the concentrations of 12.5 and 15.0%, respectively. Moreover, most embryogenic structures were formed with an osmotic potential of up to -0.691 MPa at 2.5% PEG 6000, whereas the somatic embryos showed a greater response under a lower intensity potential, mainly between -0.374 and -0.534 MPa in the media gelled with Phytagel and in agar, respectively, without

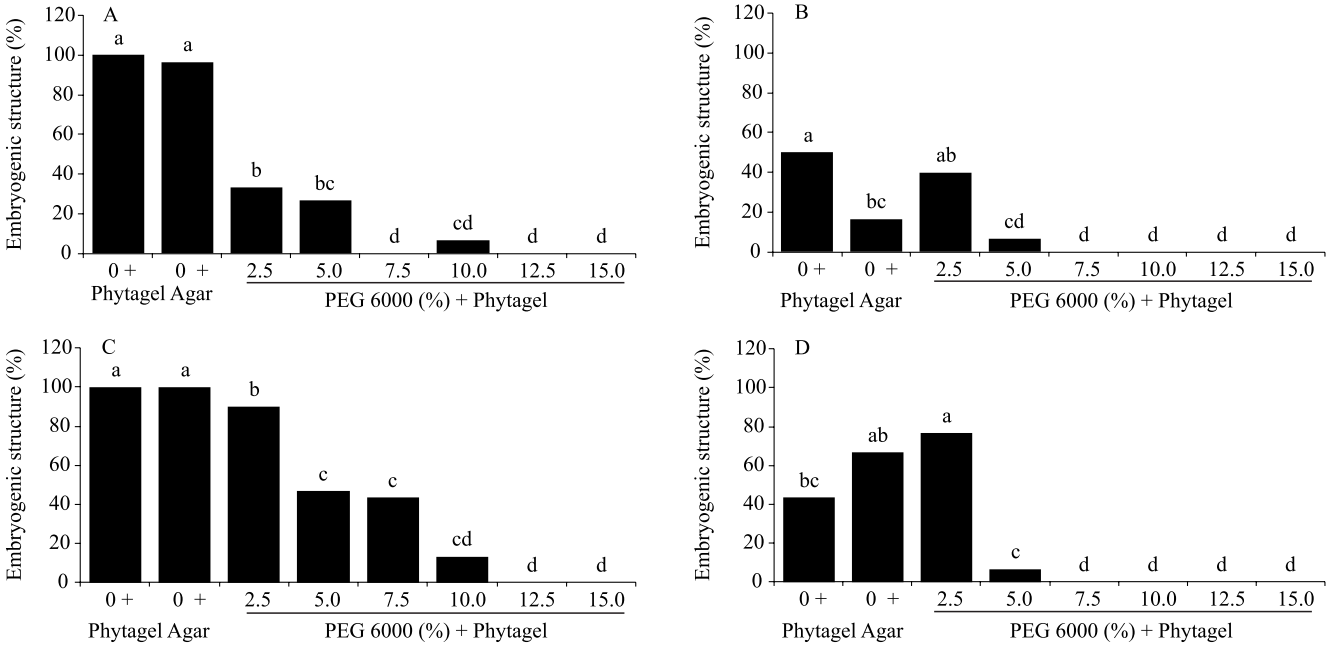

Figure 2. Effect of different concentrations of polyethyleneglycol 6000 (PEG 6000) on the percentage of embryogenic structures formed in the leaf explants of four coffee (*Coffea Arabica*) genotypes cultivated in direct somatic embryogenesis medium gelled with 2 g L^{-1} Phytagel or 5 g L^{-1} agar, in the absence of light and at 25ºC, after 120 days from the beginning of the experiment. For each genotype, equal letters above bars show that means do not differ by Tukey's test, at 5% probability

the addition of PEG 6000. This shows that the intensity of the osmotic potential affects differently the events of the direct route for coffee explants.

The obtained results are indicative that the condition necessary for the occurrence of direct somatic embryogenesis in the studied coffee species is a low-intensity negative osmotic potential (less than -0.691 MPa), since a higher intensity leads to a more negative, reduced, or inhibited occurrence. Neofiti et al. (2020) also found that, for coffee explants subjected to the direct route, a low-intensity osmotic potential of -0.6 MPa promoted the formation of somatic embryos, unlike -1.8 MPa, which reduced it.

Therefore, in the direct via the intensity of the osmotic potential not only influences the initiation of the embryogenic structures in the first 15 days of cultivation, but can also influence the subsequent formation of somatic embryos 90 days after cultivation. This way, according to its intensity, the osmotic potential affects all events in the direct route, as well as those that occur long after the beginning of cultivation. This shows the importance of knowing the osmotic potential of the culture medium used for the induction of coffee embryos via the direct route. In the present study, it was observed that a low-intensity negative osmotic potential is one of the necessary conditions for the occurrence of direct somatic embryogenesis in the species of *C. arabica*.

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